STUDIES IN THE STRUCTURE AND SYNTHESIS OF SOME NATURAL PRODUCTS

A THESIS

Presented to

The Faculty of the Graduate Division

by

Steven Jay Steindel

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the

School of Chemistry

Georgia Institute of Technology

January 1973

STUDIES IN THE STRUCTURE AND SYNTHESIS

OF SOME NATURAL PRODUCTS

Approved:

L. H. Zalkow, Chairman

G. A. Bertrand

D. S. Caine

Date approved by Chairman: 1/29/73

TO MY MOTHER AND FATHER

ACKNOWLEDGMENTS

No doctoral dissertation is the work of one person. I would like now to thank some of those who helped make this dissertation possible.

In particular, I would like to thank greatly my parents, to whom this work is dedicated. Without them, it would have been impossible.

I wish also to thank the entire faculty of the School of Chemistry for teaching me all I know during my stay at Georgia Tech both as an undergraduate and graduate student.

I would like to thank Dr. D. H. Miles, Dr. J. B. Nabors and Dr. A. R. Kalyanaraman for teaching me many of the tricks of the trade.

I wish to thank Mr. George Turner and Mr. Larry Abbey for their assistance in obtaining both routine and especially difficult mass spectra. I would also like to thank the NSF for providing funds to Georgia Tech for the purchase of a Hitachi (Perkin-Elmer) RMU-7L High Resolution Mass Spectrometer which was used in this study.

I would like to thank Mr. Gerald O'Brien and Mr. Charles Taaffe for their help in keeping our instruments functioning.

An immense amount of thanks is due to Dr. J. A. Bertrand for his help and advice on the X-ray portion of this thesis. I also wish to thank him for serving as a member of my thesis reading committee.

Much thanks is due to Dr. D. S. Caine for his advice while Dr. Zalkow was away. His help with many problems, both professional

and personal, was invaluable. Again, I wish also to thank him for serving as a member of my thesis reading committee.

Dr. L. H. Zalkow is due more than just an acknowledgment for serving as my thesis advisor during my tenure at Georgia Tech. All I can say is "Thank you."

Lastly, I would like to thank my fiancee, Stephanie, for correcting my spelling and grammar, and also for being there.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF CHARTS

GLOSSARY OF ABBREVIATIONS

Ac	acetate (CH_3CO_2)
b	broad (IR spectra)
bp	boiling point
bs	broad singlet (NMR spectra)
Bu	butyl (C_4H_9)
cal	calculated
cc	cubic centimeter
CD	circular dichroism
Col.	column
cm	centimeter
d	doublet (NMR spectra)
DMF	dimethylformamide
Et	ethyl (C_2H_5)
Fig	figure
GC	gas chromatography
gms	grams
hrs	hours
Hz	Hertz (cycles per second)
IR	infrared (spectra)
J	NMR coupling constant
kg	kilograms
l	liters

GLOSSARY OF ABBREVIATIONS (Continued)

lit	literature
m	medium (IR spectra)
m/e	mass to charge ratio
Me	methyl (CH_3)
min	minutes
mg	milligram
ml	milliliter
mm	millimeter
mp	melting point
mμ	millimicron
NA	not available
NMR	nuclear magnetic resonance (spectra)
ORD	optical rotatory dispersion
ppm	parts per million
R_f	ratio of the distance a compound travels to the distance the solvent travels (TLC)
R_t	retention time (VPC)
s	singlet (NMR spectra)
s	strong (IR spectra)
sec	seconds
sh	sharp
temp	temperature
TLC	thin layer chromatography
TMS	trimethylsilane
TNB	trinitrobenzoate

GLOSSARY OF ABBREVIATIONS (Continued)

UV	ultraviolet (spectra)
VPC	vapor phase chromatography (GC)
w	weak (IR spectra)
$w_{\frac{1}{2}}$	width at one-half height
μg	microgram
μl	microliter

NOMENCLATURE

Chapter I

The nomenclature used in this chapter is the nomenclature used in the various referenced articles and needs no further explanation.

In this chapter, as in all subsequent chapters, a Δn indicates that the double bond starts at carbon atom (n) and ends at carbon atom (n+1).

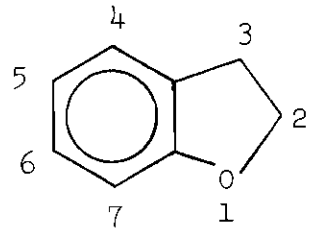
The numbering system used for the cyclic compounds is that suggested in "Reports on Symbolism and Nomenclature," J. Amer. Chem. Soc., 82, 5517 (1960). Numbering the macrocyclic ten-member eudesmane sesquiterpene precursor rings follows the above numbering. Acyclic molecules are numbered to give substituents the lowest possible numbers.

Chapter II

Nomenclature in this section is that of Simonsen and Ross.[31,47]

Chapter III

The benzofurans referred to in this section are named either as in the cited literature or as substituted derivatives of benzofuran, which is numbered as shown below:

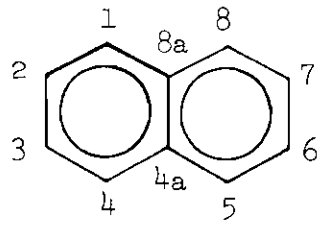


An alternate numbering scheme, forced by the need to identify all atoms in the molecule, was used in the X-ray study. That numbering system is shown on page 53 .

Absolute configurations are given by the method of Cahn, J. Chem. Ed., 41, 116 (1964).

Chapter IV

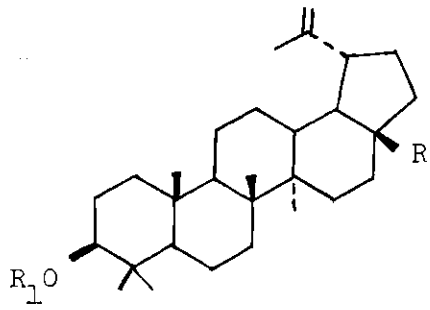
All molecules in this section are named on the basis of naphthalene derivatives, where the naphthalene is numbered as shown below:

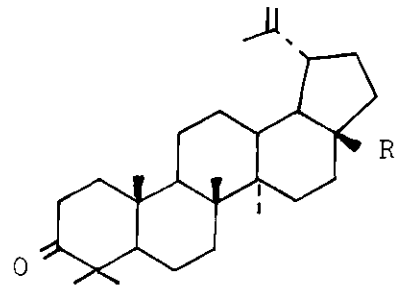


In the discussion section, an abbreviated form of the name is given, whereas the full name is given in the experimental section. For the abbreviated name, the above numbering system is used. Free use is made of the Δ nomenclature referred to in Chapter I. α refers to substituents above the plane of the molecule and β refers to substituents below the plane of the molecule.

SUMMARY

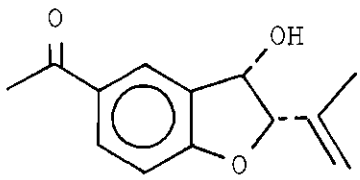
An investigation of the KB active fraction of Sarracenia flava has been undertaken. The major constituent has been isolated and shown to be betulinic acid (1).[97] Antitumor testing by the National Cancer Institute of it and several of its derivatives (2 - 5) showed that all have no antitumor activity.



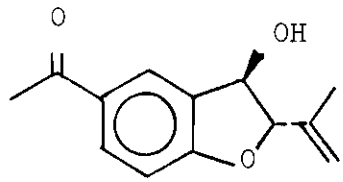


1. R = CO_2H, R_1 = H
2. R = CO_2CH_3, R_1 = H
3. R = CO_2H, R_1 = $COCH_3$
4. R = CO_2H
5. R = CO_2CH_3

An X-ray structure analysis of the compound reported to be trans-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran[37] has shown it to be really the cis isomer. These results caused a revision in the structure of toxol from (6)[36] to (7).

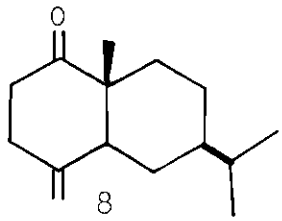


6

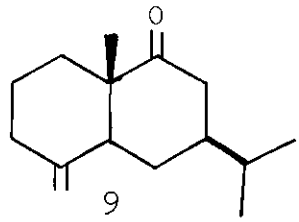


7

Canarone has been reported to have both structures (8)[24] and (9). Synthesis of (9) has shown that it was different from canarone.[95] We wish to report the synthesis of (8) as its enantiomer and comment on

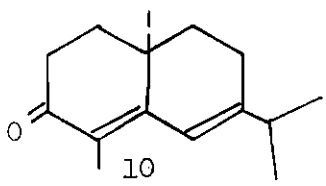


8

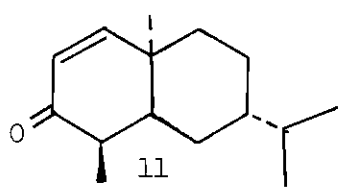


9

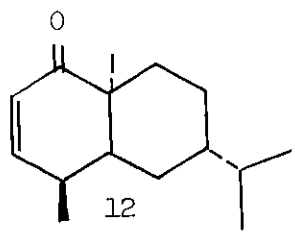
its spectral differences from canarone. Synthesis was accomplished by using the McQuillin synthesis of β-cyperone (10),[97] which was converted in five steps to α,β-unsaturated ketone (11). Conversion of (11) to an isomeric α,β-unsaturated ketone (12) was accomplished in two steps. Ketone (12) was converted into the enantiomer of (8) through its deconjugated ketal. Epoxidation of the ketal, followed by reduction with lithium aluminum hydride, gave alcohol (13) which



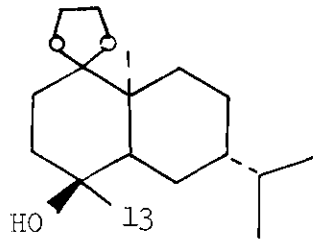
10



11



12



13

was readily dehydrated to the enantiomer of (8). Attempts to convert (12) to the enantiomer of (8) by several other methods failed to yield the desired results.

CHAPTER I

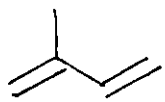
INTRODUCTION

Natural products chemistry, a small portion of which this dissertation treats, is in many respects the starting and meeting point of the vast field of organic chemistry. Indeed, one can view organic chemistry as getting its first real beginning when Wöhler synthesized urea, a natural product, in 1828 and showed that no "vital force" was needed to synthesize an organic compound.[1] Isolation and structure determination of many natural products then followed, yielding much information for future chemists. However, the era of total synthesis did not begin until the 1930's, one hundred years after Wöhler's work.

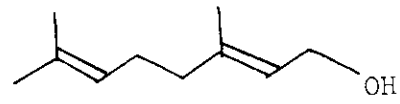
R. B. Woodward, one of the great masters of the art of total synthesis,views the field in the following manner:[2] "There is excitement, adventure, and challenge, and there can be great art, in organic synthesis." One should view natural products chemistry in this light. When one reads a paper concerning the total synthesis of vitamin B_{12} by Eschenmosher and Woodward[3] one has to feel the awe inspired by humans accomplishing an immense task, similar to the feeling of viewing a large elaborate Renaissance painting like DaVinci's "Last Supper" or Michelangelo's Sistine Chapel. The work of E. J. Corey seems light, airy and simple, but with much hidden meaning, like a painting of Monet or Van Gogh. The works of G. Stork and G.

Büchi seem similar to their western European background in that they are precise, elaborate, complete works, like a painting by Rembrandt. It is truly unfortunate that the world at large cannot pass judgment on chemical syntheses as works of art, for indeed they are.

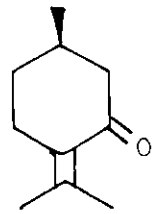
However, this dissertation is not in art history, but in natural products chemistry, and the discussion must now turn back to chemistry. Primarily, we are concerned with the chemistry of the natural products collectively referred to as the terpenes. Terpenes are compounds which follow the general formula $(C_5H_8)_x$, with many oxygenated derivatives known. Classification of terpenes is based on the number of isoprene units in the structure.[4] Hemiterpenes contain one isoprene unit, and isoprene (I-1) is the only member. Monoterpenes (sometimes referred to as terpenes) contain two isoprene units and exist in



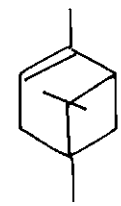
I-1



I-2



I-3



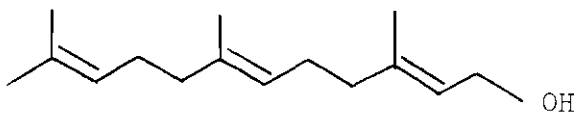
I-4

acyclic (e.g., geraniol[5](I-2)), monocyclic (e.g., pulegone[6](I-3)), and dicyclic (e.g., α-pinene[7](I-4)) forms. Sesquiterpenes contain three isoprene units and their structural complexity increases greatly. Corey has commented on the interest and variety in

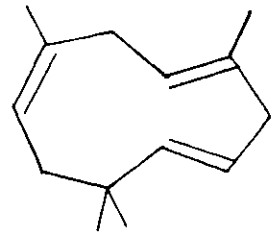
sesquiterpene structure in the following manner:[8]

> Here (referring to sesquiterpene structure) diverse and unusual arrangements of rings and functionality abound, notwithstanding a common origin from the same acyclic C-15 precursor, as now seems probable. This remarkable variety of design is perhaps the principal reason for the structural chemist's deep interest in this field of natural products. . .

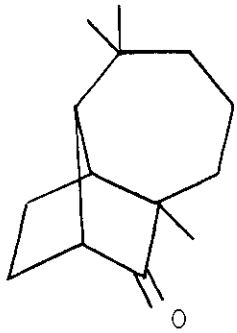
Some common sesquiterpenes are farnesol (I-5),[9] humulene (I-6),[10] longifolene (I-7),[8,11] sesquicarene (I-8),[12] β-eudesmol (I-9),[13] intermedeol (I-10),[14] bulensol (I-11),[15] elemol (I-12),[16] eremophilone (I-13),[17] and acoreone-B (I-14).[18] The above brief list does not do justice to the vast amount of work done in the field of sesquiterpene chemistry, but it does lend credence to Corey's statement. Continuing onward we have the diterpenes (four isoprene units) whose structural complexity we shall not discuss. Our next broad class of



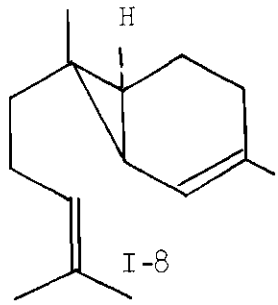
I-5



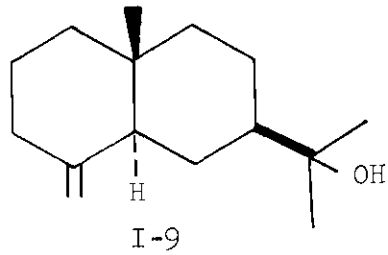
I-6



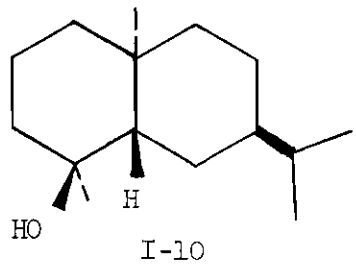
I-7



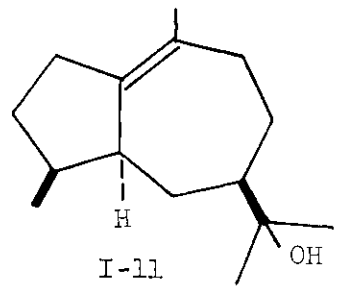
I-8



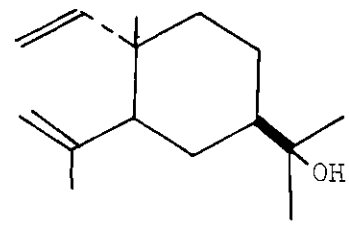
I-9



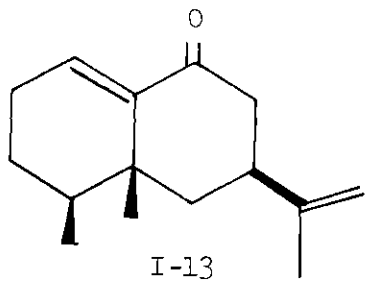
I-10



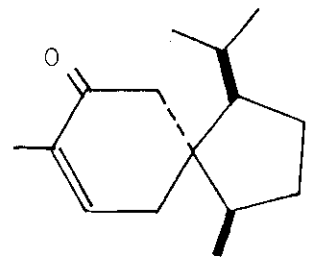
I-11



I-12

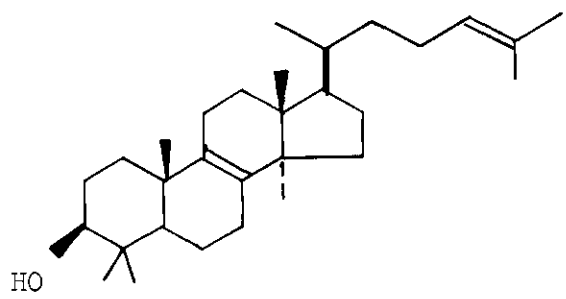


I-13

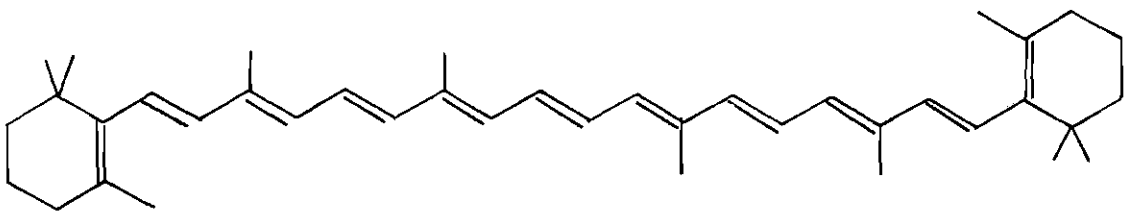


I-14

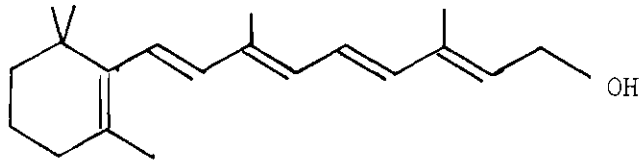
terpenes are the C-30 triterpenes and their first cousins, the steroids, which are derived from lanosterol (I-15).[19,20] Finally, the last class of terpenes is the polyterpenes, which includes such diverse compounds as natural rubber and the carotenes (β-carotenone (I-16) and vitamin A alcohol (I-17)).[21]



I-15

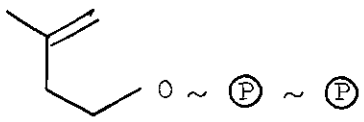


I-16

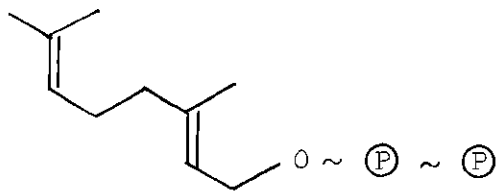


I-17

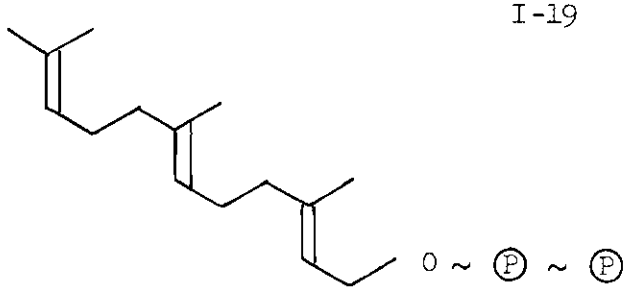
The diverse compounds referred to above as the terpenes are grouped together because a common biogenetic precursor has been found. All of the above are thought to be produced in some way from isoprene as a precursor.[4] Biogenetic isoprene (I-18) is prepared from acetyl coenzyme A by a series of simple transformations.[22]



I-18



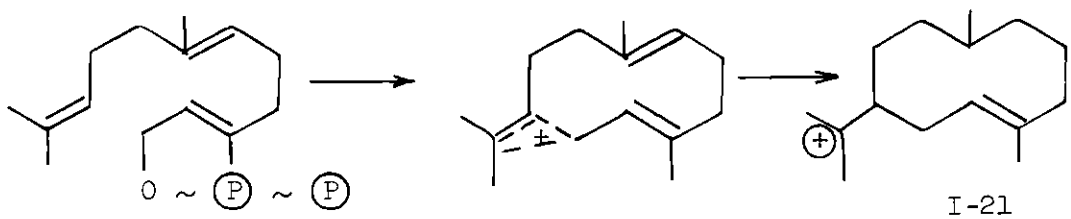
I-19



I-20

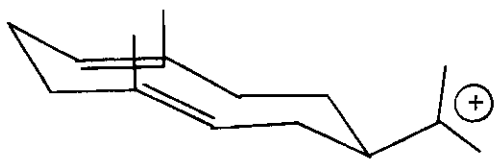
Enzymatic coupling of two isoprene units given geranyl pyrophosphate (I-19), which is the precursor for all monoterpenes. Further enzymatic coupling of geranyl pyrophosphate with biogenetic isoprene gives farnesyl pyrophosphate (I-20), which is the precursor to the sesquiterpenes. Humulene (I-6) can be derived from (I-20) by simply cyclizing about the ends of the molecule. Humulene and longifolene (I-7) are related by a C-1 to C-10 hydride shift, and then appropriate cyclizations. Sesquicarene involves first cyclization of (I-20) to a six-membered ring, then rearrangement to give the fused cyclopropane ring.

The eudesmane sesquiterpenes arise in the following manner:

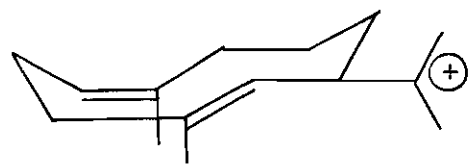


I-21

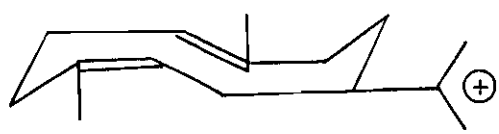
One can view (I-21) as folding in one of three possible conformations, chair-chair (I-22), boat-chair (I-23) and boat-boat (I-24).[22,23]



I-22

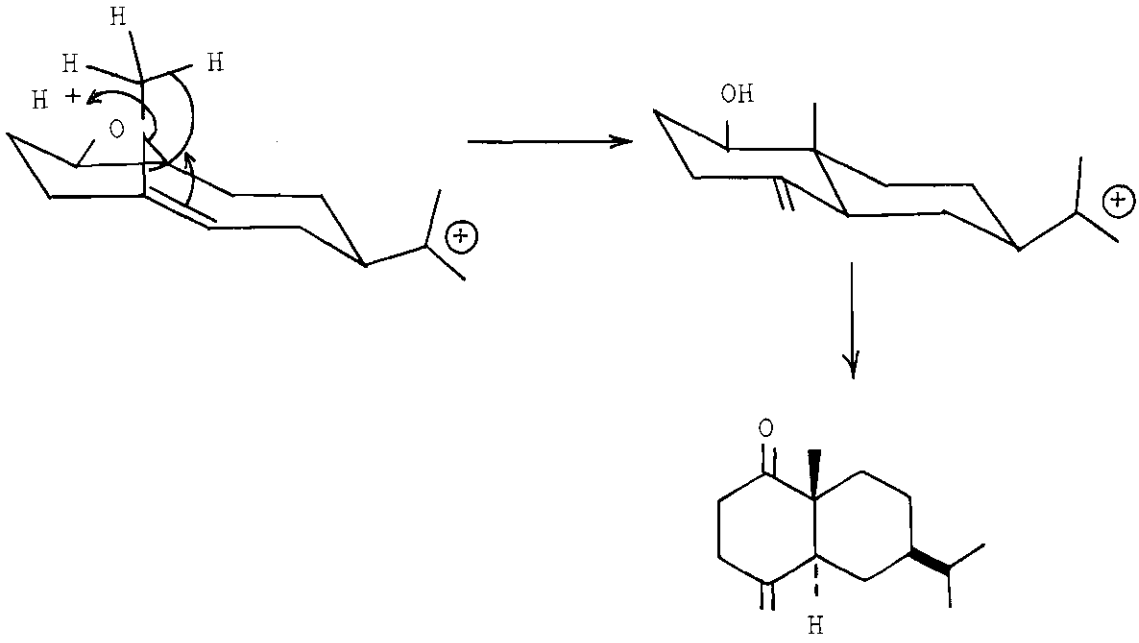


I-23



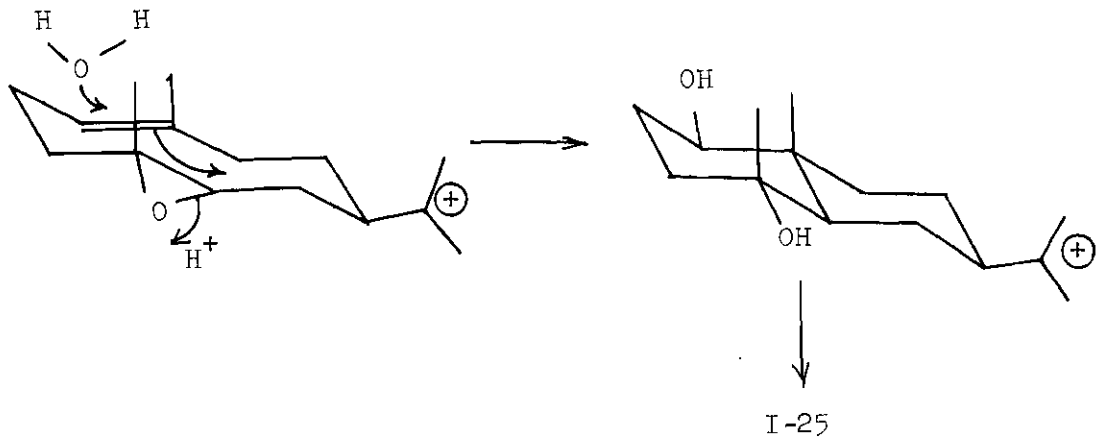
I-24

Eudesmanes from each of these possible foldings are known. If we epoxidize the Δ^4 double bond and cyclize the chair-chair form by attack of H^+ in the usual manner with concerted ring closure, we are left with the normal eudesmane products like β-eudesmol (I-9). Similarly, if we epoxidize the Δ^1 double bond and cyclize (see below) we are left with C-1 oxygenated eudesmanes. For this concerted



I-25.

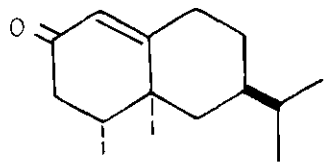
mechanism the hydrogens on the methyl at C-4 are aligned correctly for formation of the exocyclic double bond. One need not epoxidize between C-1 and C-10 to give this product, but can use the previously mentioned C-4 - C-5 epoxide:



I-25

In both cases, the final eudesmane structure drawn is that of canarone (I-25),[24] whose structure is in doubt. Whether the above structure is correct for canarone is unknown, but the existence of a eudesmane sesquiterpene of this structure is biogenetically quite reasonable. All known "normal" eudesmane sesquiterpenes (arising from the chair-chair folding) can be derived from either the C-1 - C-10 epoxide or the C-4 - C-5 epoxide. It is unlikely, but possible, that nature has chosen both pathways by which to make these compounds; however, the correct one is at present unknown. Cyclization of the boat-chair (I-23) folding in a similar manner to that above gives eudesmane sesquiterpenes with the intermedeol (I-10) stereochemistry. The eremophilane sesquiterpenes are thought to arise via angular methyl migration.[25] Migration from the chair-chair-produced eudesmanes gives the eremophilane stereochemistry (I-13). The oxygen at C-9 is of interest. From the above pathway one can place oxygen at C-1 or C-4 in the eudesmane skeleton directly. One can only place oxygen at

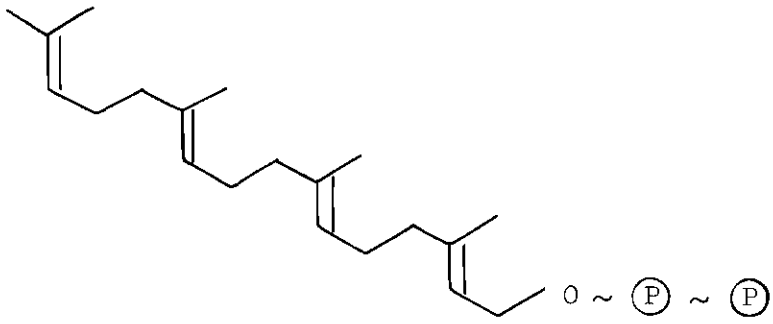
C-9 by some allylic oxidation or similar pathway. It is felt that the oxygen at C-9 in eremophilone arises from an allylic oxidation after methyl migration and formation of the double bond. Methyl migration in the case of boat-chair-formed eudesmanes leads to the valencene-type compounds like nootkatone (I-26).[26] Proof of this



I-26

postulated methyl migration is unavailable and laboratory attempts to duplicate it have failed.[27] In a similar manner all the sesquiterpenes mentioned previously can be derived from farnesyl pyrophosphate.

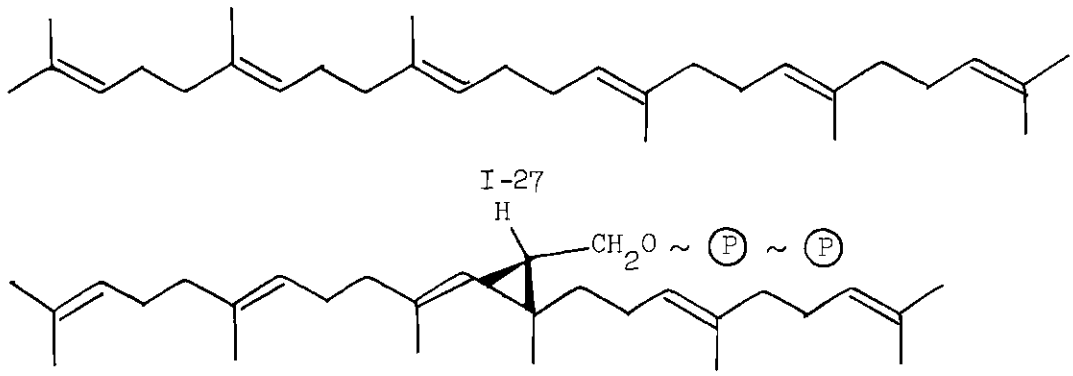
Linkage of farnesyl pyrophosphate (I-20) with Δ3-isoprene pyrophosphate (I-18) gives geranylgeranyl pyrophosphate (I-26) which leads



I-26

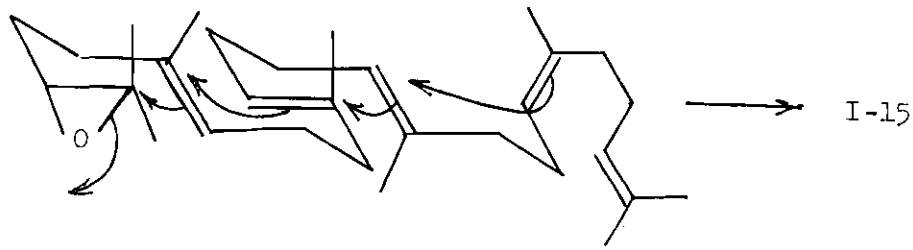
to the diterpenes.[4] Dimerization of geranylgeranyl pyrophosphate gives the carotenoids.[28]

Dimerization of farnesyl pyrophosphate to squalene (I-27), through pre-squalene (I-28),[29] and then cyclization leads to the

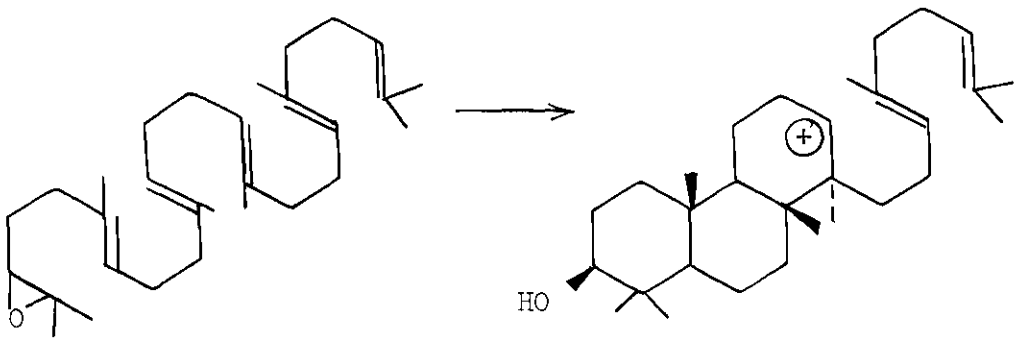


I-28

triterpenes. Enzymatic cyclization of squalene 2,3-oxide[19] yields lanosterol (I-15).[20] The following folding of squalene oxide with the appropriate cyclization is the mechanism by which this is thought to occur:

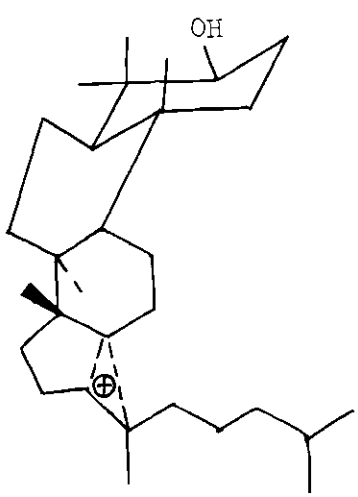


Lanosterol is enzymatically demethylated to give the steroid carbon skeleton. The triterpenes can be derived from squalene 2,3-oxide in the following manner:[20]

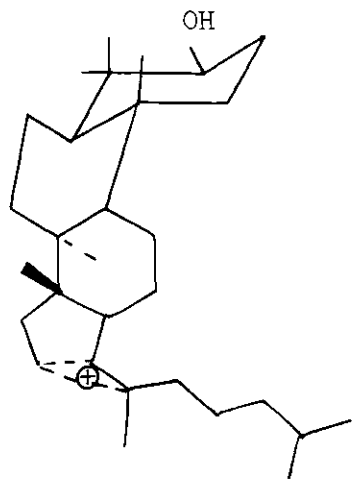


I-29

Carbonium ion (I-29) can be viewed biogenetically as a bridged ion existing in several forms, two of which (I-30) and (I-31) are shown below:



I-30

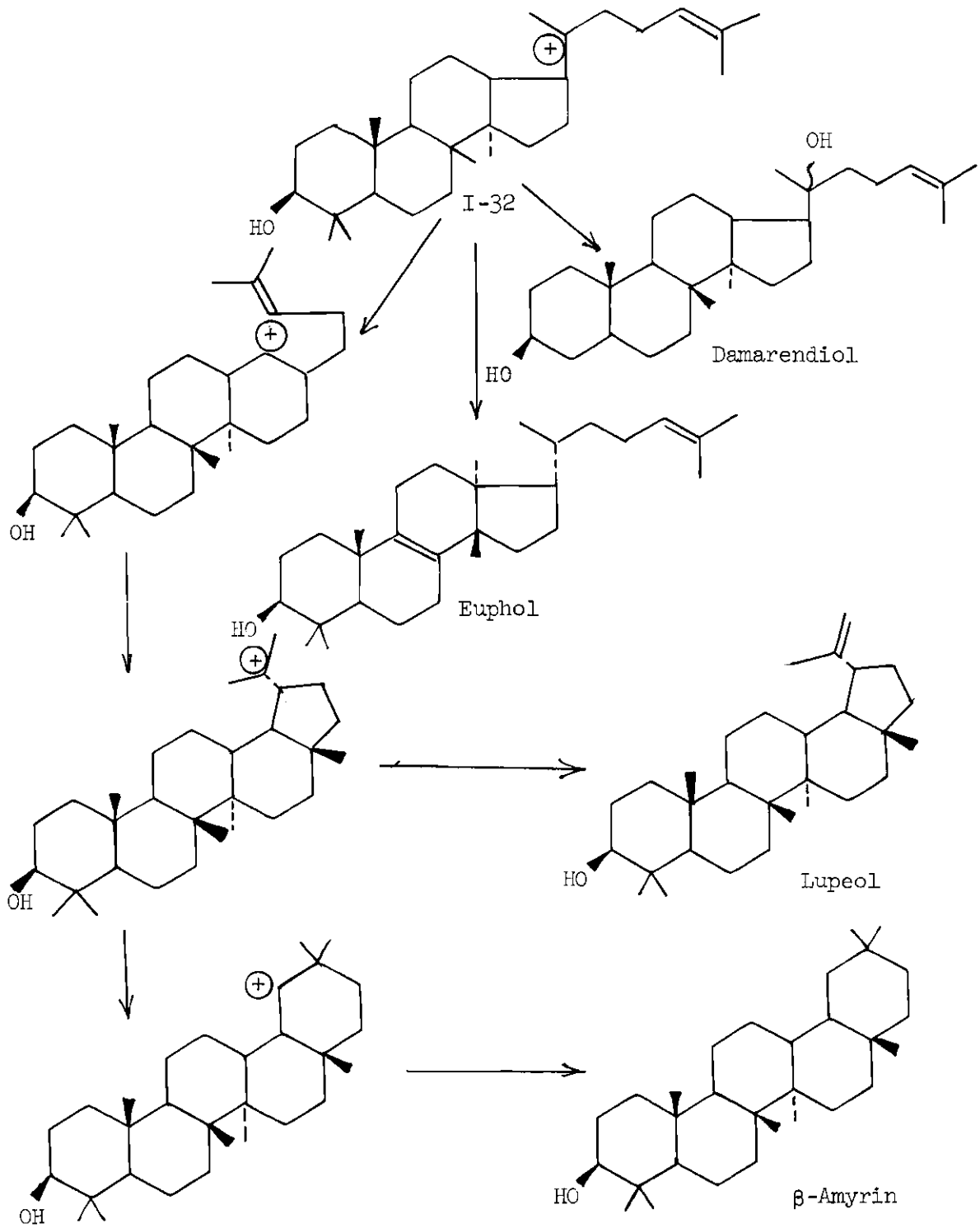


I-31

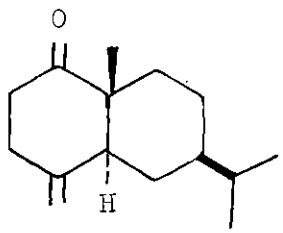
Or it can be viewed as a localized carbonium ion (I-32). Whatever form it exists in, the rearrangements shown in Chart 1 are possible. Recently, this conversion from squalene 2,3-oxide to the triterpene carbon skeleton has been done in vitro.[30]

Chart 1

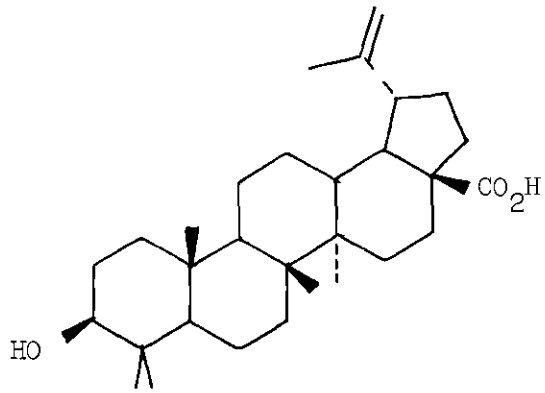
Biogenesis of Triterpenes



In this study we shall concern ourselves with two compounds, one a sesquiterpene of the eudesmane type, canarone (I-25),[24] and a triterpene of the lupeol family, betulinic acid (I-33).[31] These compounds have both been isolated previously. In the case of betulinic



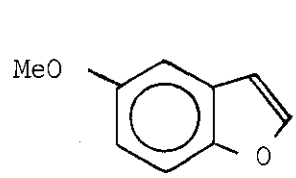
I-25



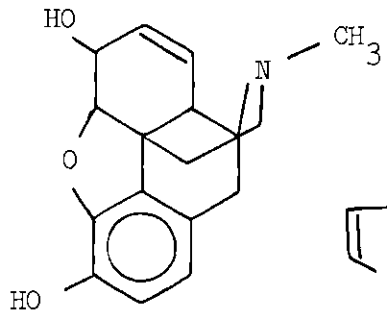
I-33

acid we have confirmed its presence in a carnivorous pitcher plant, Sarracenia flava, by comparison with the known properties of the acid and finally by direct comparison of its reduction product with betulin. For canarone we have attempted to confirm the proposed structure by synthesis in an unambiguous manner, a standard practice in natural products chemistry.

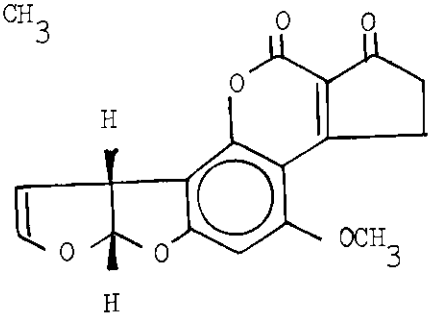
Lastly we turn our attention to another class of natural products, the benzofurans. The benzofuran functionality is found in many diverse molecules such as 5-methoxybenzofuran (I-34)[32] isolated from oak beer barrel fungal contamination, morphine (I-35)[33] from opium poppies, and aflatoxin B_1 (I-36),[34] a potent carcinogen isolated from Aspergillus flavus. Other interesting and diverse



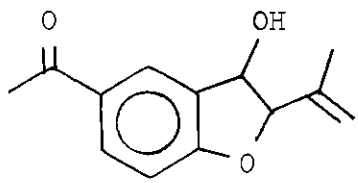
I-34



I-35



I-36



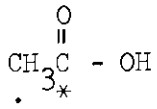
I-37

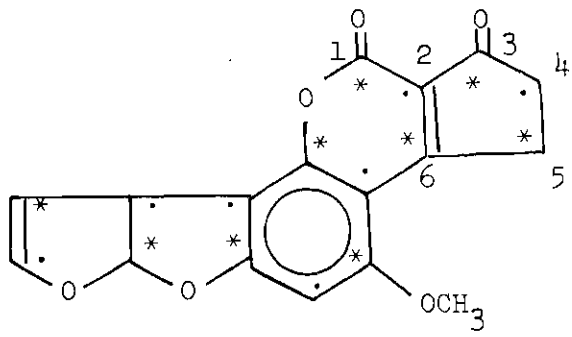
benzofurans have been reviewed by Dean.[35] We are concerned in this research with the structure of toxol (I-37), isolated by Zalkow and Burke.[36] Of primary interest is the stereochemistry of the OH to the isopropylene group, which has been reported to be cis. The reported structure has been revised by inferring the correct structure of toxol from an X-ray study of a synthetic intermediate prepared during the synthesis of dihydrotoxol.[37]

From a biogenetic viewpoint, not much is known about how benzofurans are constructed. Büchi has studied the biosynthesis of aflatoxin B_1 using ^{14}C labelled acetate.[38] Other workers had shown that mevalonate was not the precursor to the aflatoxins, ruling out a pathway similar to the terpenes. Büchi's results are summarized below. The molecule is probably constructed biogenetically from

several polyacetate chains.

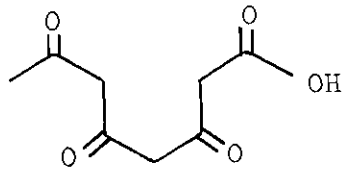
Key:



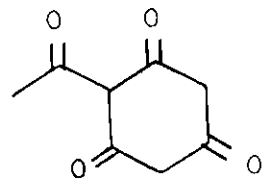


I-38

One chain forms furan ring A, another chain makes the benzene ring and carbon atom C-6, finally another chain is used to add C-1 to C-5. The microorganism then links these chains together and biogenetically transforms them into the required functionality for the aflatoxin. Toxol, the compound with which part of this thesis deals, can be looked upon as also deriving in part from acetate. Ramming has proposed a polyacetate chain (I-39) as a likely precursor to toxol and other benzofuran systems.[39] Cyclization to a six-membered ring follows and is a very likely precursor to the benzene ring (I-40).



I-39



I-40

At this point, the biogenetic pathway deviates from that of the aflatoxins. The furan ring of toxol is isoprenoid in nature. Since terpenoids have been isolated from rayless goldenrod[40] it is obvious that the plant has the enzymatic capacity to generate the required terpenoid precursor. Hence, it appears most reasonable to assume that the acetoxybenzene portion of toxol is derived from acetate and the furan ring from biogenetic isoprene (I-18) as suggested by Ramming.[39]

During the course of this work we have studied the structures of three natural products by three of the four commonly used techniques of natural products chemistry. The first is direct comparison of the properties of an isolated natural product with those of one previously reported. The second is determination of the structure of another natural product by inference using known information combined with new information. The third technique is the use of synthesis to study the structure of still another natural product. The fourth method, direct determination of the structure of a new natural product, was not used. Usually this is done by direct method X-ray crystallography, or for simple molecules, spectral methods. For this work, there was no need to use this method. Let us now see the results of our study.

CHAPTER II

BY COMPARISON: ISOLATION OF BETULINIC ACID FROM SARRACENIA FLAVA

Introduction

The ethanol (moonshine) extract of the roots of Sarracenia flava (golden trumpet), a carnivorous pitcher plant found in the Okefenokee Swamp in southeastern Georgia, has been used as a folk remedy by the inhabitants of that region.[41] A study was undertaken in order to find out if any chemicals in the plant are of medicinal value.

Preliminary methanol extraction of the plant's roots, which were collected in the area around the Okefenokee Swamp during the month of February, yielded two fractions, a methanol-soluble oil and a methanol-insoluble, beige-colored solid.[42] Testing of these crude fractions by the National Cancer Institute (NCI) showed that against human epidermoid carcinoma of the nasopharynx (KB) a dose of 27 μg/ml of the oil was required to inhibit 50 percent growth of the test cells versus the control (ED_{50}), while in the same system the solid showed an ED_{50} of 11 μg/ml. The solid was judged to warrant further study by the NCI and showed in the later tests ED_{50}'s of 3.0 μg/ml, 2.6 μg/ml, and 2.6 μg/ml. These tests confirmed KB activity and further investigation was justified. Neither sample was active against L-1210 lymphoid leukemia in BDF_1 mice or Walker carcinosarcoma

256 (Intramuscular) in random bred albino rats.

More plant material was collected during the month of May in an area just outside the Okefenokee Swamp. Extraction of the finely ground roots was carried out using the Wall procedure for KB active compounds (see Chart 2).[43,44] Testing was then performed by the NCI on several of the indicated fractions. The results are shown in Table 1. It is clear that the KB activity is now concentrated in Fractions II and V, but it is also clear that the activity of the fractions is much less than that observed from preliminary extraction.

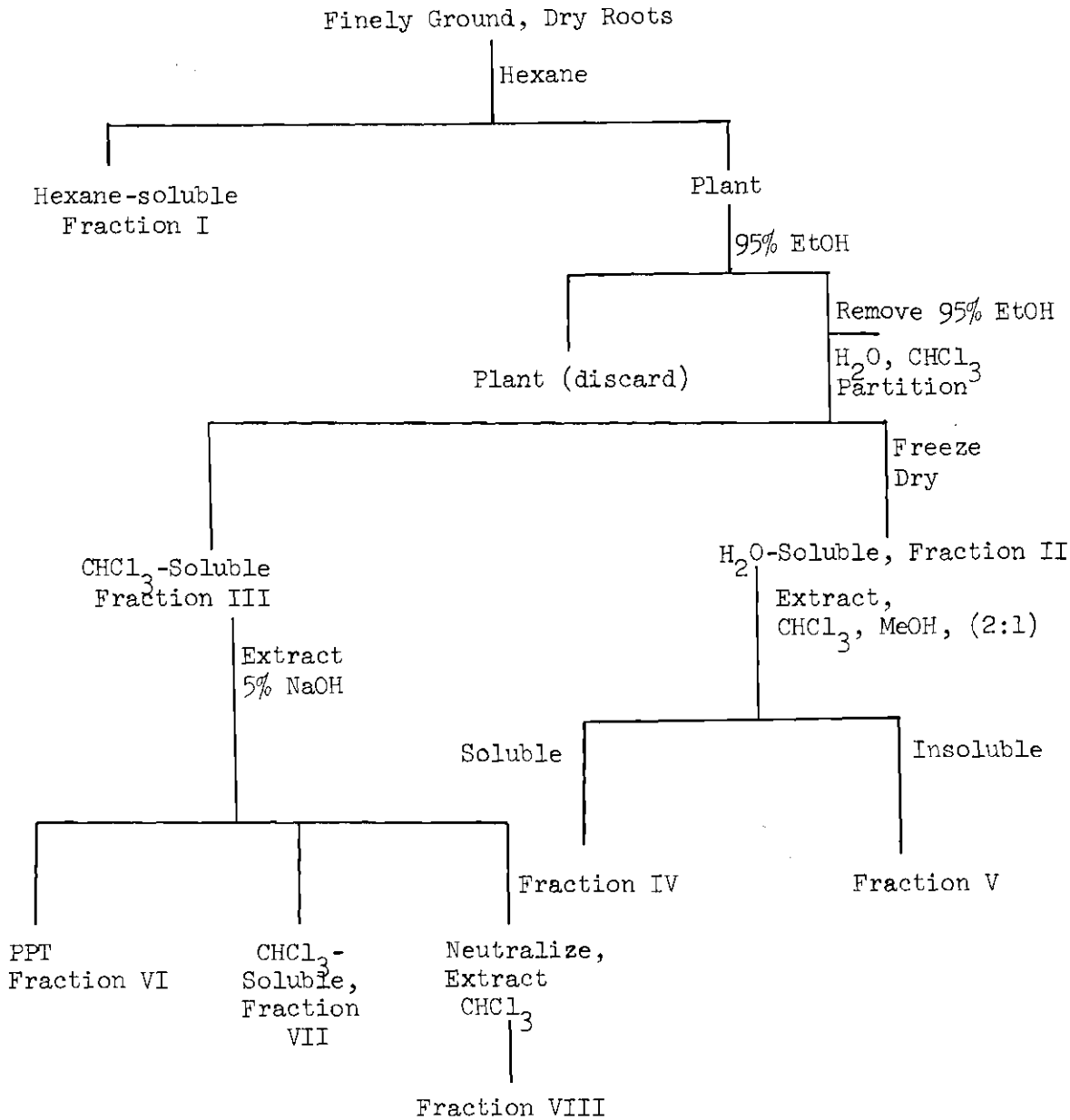
Table 1. Activity of Fractionated Plant Material.

Fraction No.[a]	% Plant Mat.	ED_{50}[b]	Slope
I	7.9	>100	0.0
II	13.6	26	-1.09
III	2.3	>100	0.0
IV	2.0	>100	0.0
V	0.3	24	-0.51
VI	12.5	NA	
VII	1.0	NA	
VIII	0.2	NA	

[a] See Chart 2

[b] Activity against human carcinoma of the nasopharynx, *in vitro*, expressed as μg/ml that inhibited 50 percent of control dose. Slope is the change of response for each one-log change of dose.

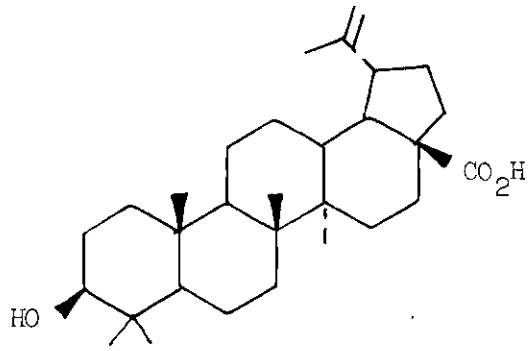
Chart 2. Extraction by Wall Procedure



A study was undertaken of the beige-colored solid isolated in the methanol extraction and also Fraction VI from the extraction performed according to the Wall procedure in order to see if the antitumor agent could easily be isolated from them.

Experimental

Isolation of Betulinic Acid



The beige solid Fraction VI (5.1 gms) was recrystallized from hot methanol-acetone to yield a white crystalline material (3.7 gms) which did not melt when heated to 315°. The salt (2.9 gms) was partially dissolved in 10 percent HCl and the acid layer was continuously extracted with $CHCl_3$. After all the solid had dissolved, the $CHCl_3$ was dried over $MgSO_4$ and removed *in vacuo* to yield an off-white solid which could be recrystallized from ether (1.5 gms). Mp 290-292°, reported[31] for betulinic acid 316-318°.

IR: $\nu_{KBr}(cm^{-1})$:~3500 - ~2500 (b,ROH and RCO_2H), 2925 (s), 2850 (m), 1690 (s), 1650 (m).

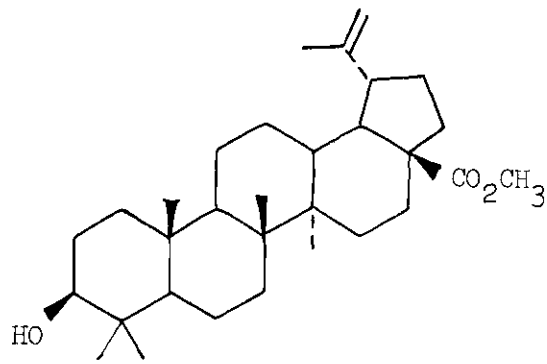
NMR: not taken because of solubility problems.

Mass Spectrum: M^+ = 456 (100%, base peak), M^{+1} = 25%, M^{+2} = 5%.

Recrystallization of the beige-colored solid isolated in the initial methanol extraction from hot methanol yielded betulinic acid as the only identifiable product.

Preparation of Betulinic Acid Methyl Ester

The above recrystallized acid (1.5 gms) was suspended in MeOH. To this was added a solution of ~0.2 moles of diazomethane prepared from EXR-101® in a ground-glass-free apparatus. The diazomethane addition was repeated to ensure complete reaction. After all the diazomethane had evaporated, the solvent was removed *in vacuo* to yield 1.4 gms of methyl ester. Recrystallization from MeOH gave a constant melting point of 220-221° (reported 224-225°).[31]



IR: $\nu_{KBr}(cm^{-1})$: 3530(sh, OH), 2930 (s), 1700 (s), 1640 (m).

NMR:(δ, $CDCl_3$): 0.75, 0.83, 0.92, 0.97 (methyl protons), 1.70 (s, 3H), 3.67 (s, 3H), 4.70 (complex, 2H).

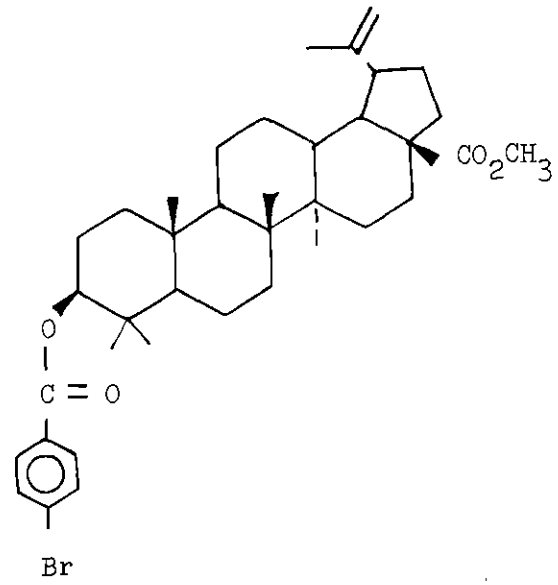
Mass Spectrum: M^+_{found} = 470.3778, calculated = 470.3759 ($C_{31}H_{50}O_3$)

Base peak (m/e) = 189.

® Registered trademark, E. I. DuPont, Inc.

Preparation of p-Bromobenzoate of Betulinic Acid Methyl Ester

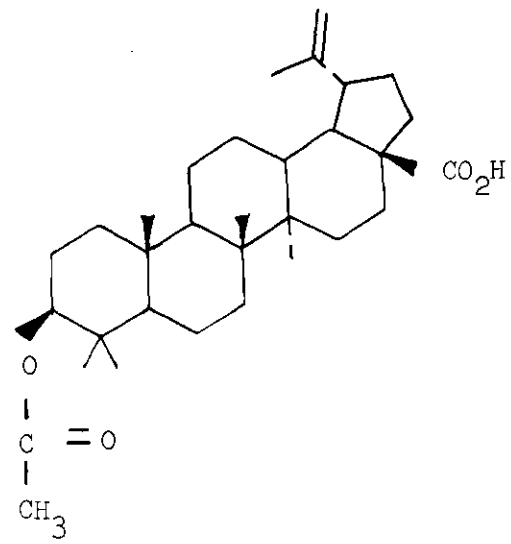
The triterpene ester (100 mg) was treated with p-bromobenzoyl chloride (50 mg) in 10 ml of dry pyridine for 24 hours at room temperature. Then the solution was poured into crushed ice and extracted with ether. The ether layer was washed with 10 percent HCl until acidic, dried over $MgSO_4$, and removed in vacuo to yield 139 mg of the p-bromobenzoate of betulinic acid methyl ester, mp = 184 - 185°.



IR: $\nu_{KBr}(cm^{-1})$: 1725 (s), 1675 (s), 1635 (m), 1580 (sh), 770 (sh).

Preparation of Betulinic Acid Acetate

Freshly recrystallized betulinic acid, isolated from Fraction VI, was dissolved in a one-to-one mixture of acetic anhydride and dry pyridine. The reaction mixture was stirred at room temperature overnight, then poured into ice-water. Extraction with 10 percent HCl, then 5 percent



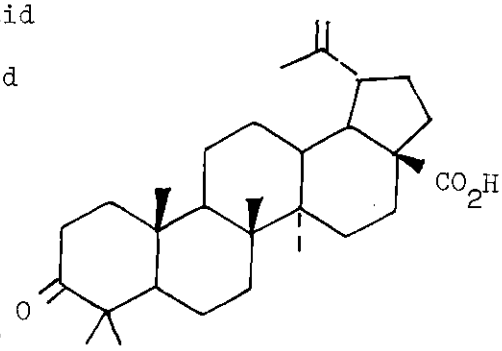
$NaHCO_3$, drying over $MgSO_4$, and then removal of the solvent in vacuo yielded the acetate of betulinic acid. Mp = 258 - 261° (reported 290 - 292°).[31]

IR: $\nu_{KBr}(cm^{-1})$: ~3500 - ~2500 (b, acid OH), 1725 (s), 1690 (s), 1640 (m), 1240 (s).

Mass Spectrum: M^+ = 498.3692, calculated = 498.3709 ($C_{31}H_{50}O_4$); base peak (m/e) = 189.

Preparation of Betulonic Acid

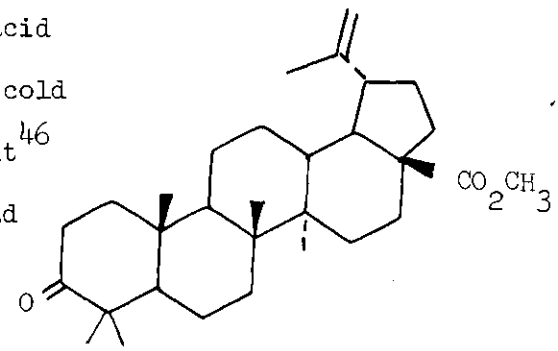
Freshly recrystallized betulinic acid was dissolved in cold acetone and titrated with Jones reagent[46] until an excess was indicated. The chromium salts were removed by filtration and washed several times with ether. Removal of the solvent in vacuo left betulonic acid, mp = 225 - 228° (reported 253°).[47]



IR: $\nu_{KBr}(cm^{-1})$: ~3500 - ~2500 (b, acid OH), 1725 (s), 1700 (s), 1640 (m).

Mass Spectrum: M^+ = 454, calculated = 454 ($C_{30}H_{46}O_3$); base peak (m/e) = 218.

Preparation of Betulonic Acid Methyl Ester



The methyl ester of betulinic acid previously prepared was dissolved in cold acetone and reacted with Jones reagent[46] as above. The isolated keto-ester had a mp = 168 - 170° (reported 165°).[47]

IR: $\nu_{KBr}(cm^{-1})$: 1725 (s), 1700 (s), 1640 (m).

Mass Spectrum: M^+ = 468.4906, calculated = 468.3681 ($C_{31}H_{49}O_3$)

base peak (m/e) = 189.

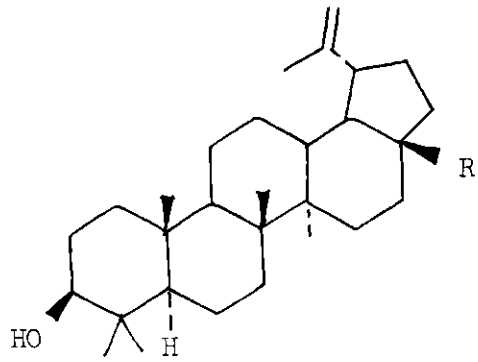
Antitumor Testing:

The above acid, methyl ester, acid-acetate, keto-acid and keto-ester were sent to the National Cancer Institute and were tested for antitumor activity in the usual manner. The results are shown in the discussion section.

Results and Discussion

Preliminary investigation of Fraction VII yielded, after chromatography on neutral alumina, a white crystalline solid, mp = 214 - 217°, $[\alpha]_D$ = +34.8° (pyridine).[44] An exact mass determination showed an M^+ = 442.342, which corresponded to $C_{30}H_{50}O_2$ (calculated M^+ = 442.381). A small portion of the compound was forwarded to Dr. R. L. Hale in Dr. C. Djerassi's laboratory at Stanford University who ran a mass spectrum and compared it with those in their vast files. He concluded that the compound was similar to betulin (II-1)[44,49] (mp = 252 - 253°, $[\alpha]_D$ = +20° (pyridine)).[48] Dr. Hale's

spectrum has since been reproduced at Georgia Tech and thin layer chromatography showed that the original compound had at least two spots, the major one having an R_f value which was identical with that of betulin (obtained from the Aldrich Chemical Company). The presence of the second compound readily explains the differences between the



II-1: R = CH_2OH
II-2: R = CO_2H

physical properties we have observed and those reported. These results led us to suspect the presence of other lupane-type triterpenes in the plant roots.

Hence, we investigated the salt that had precipitated upon base extraction. Acidification, followed by continuous chloroform extraction, yielded a carboxylic acid with the molecular weight of betulinic acid (II-2). Our observed mp was low (observed 290-292°), reported 316 - 318°),[31] but at this time it had not been proven that betulin was present, and we thought the stereochemistry of one of the centers in our triterpene alcohol was different from betulin. It was decided that an X-ray structure analysis of a heavy atom

derivative was in order. The p-bromobenzoate of the methyl ester of the triterpene acid was prepared and crystals suitable for X-ray analysis were grown. Analysis of the photographs taken for space group determination was not undertaken since the structure was found by another method.

At this time Dr. J. B. Nabors showed that the reduction of the acid with lithium aluminum hydride yielded a diol whose infrared, NMR and mass spectra were identical with those of betulin.[49] We feel that this experiment conclusively proves the identity of the acid as betulinic acid.

Recrystallization of the beige-colored solid that had formed during the initial methanol extraction showed that the only identifiable compound was also betulinic acid. Studies by Dr. D. H. Miles at Mississippi State University showed that betulinic acid makes up 3.4 percent of the plant material and 72 percent of the chloroform-soluble fraction.[50]

The large percentage of betulinic acid in this fraction led us to suspect that it was the agent responsible for the KB activity of the extract. Samples of betulinic acid (II-2), its methyl ester (II-3), the acid-acetate (II-4), its keto derivative (betulonic acid (II-5)), and the keto-ester (methyl betulonate (II-6)) were sent to the National Cancer Institute for antitumor testing. The results are displayed in Table 2 . It is clear from this data that betulinic acid is not the active constituent of the plant. Since betulinic acid is the major constituent of the active material and it is not

Table 2. Activity of Betulinic Acid and Some Related Compounds[a]

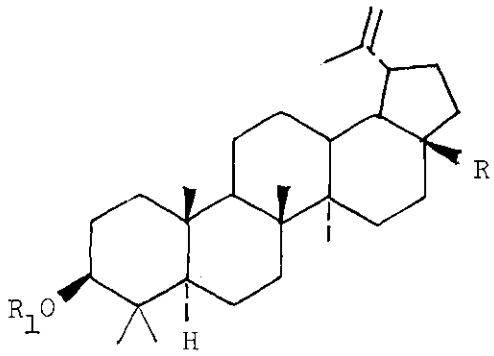
Compound	Dose (mg/kg)	T/C
Betulinic Acid (II-2)[b]	400	76
	200	90
	100	101
Betulinic Acid (II-2)[c]	200	91
	100	91
	50	101
Methyl Betulinate (II-3)[d]	400	91
	200	100
	100	103
Acetobetulinic Acid (II-4)	400	91
	200	93
	100	93
Betulonic Acid (II-5)	400	94
	200	96
	100	102
Methyl Betulonate (II-6)	400	94
	200	96
	100	91

[a] All tests on L-1210 lymphoid leukemia in BDF_1 strain of mice using intraperitoneal injections with hydroxypropylcellulose (HPC, Klucel) as a vehicle unless otherwise noted. T/C is the ratio of the mean survival time of the test animals to the control animals.

[b] Test on Walker carcinosarcoma 256 (subcutaneous) in random bred albino rats using intramuscular injections, with carboxymethyl cellulose as a vehicle.

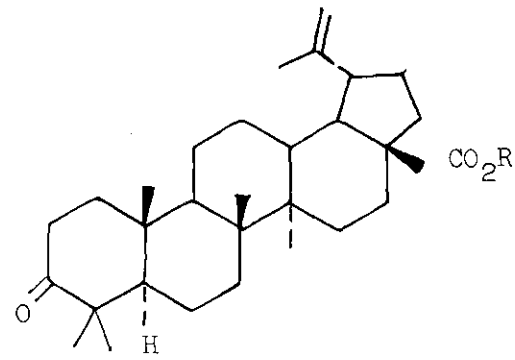
[c] Test on P-388 lympholytic leukemia in BDF_1 strain of mice. Injections were intraperitoneal using saline with Tween-80 as a vehicle. Activity against KB cell culture showed an ED_{50} of 26 and a slope of -1.16.

[d] Alcohol used as a vehicle.



II-3: R = CO_2CH_3, R_1 = H

II-4: R = CO_2H, R_1 = $COCH_3$



II-5: R = H

II-6: R = CH_3

active, it is felt that the active component may prove to be very potent. Studies are now under way at Mississippi State University to isolate and identify this compound.

CHAPTER III

BY INFERENCE: THE STRUCTURE OF TOXOL

Introduction

"Milk sickness" was a prevalent disease in the sparsely populated regions of the American Mid-west throughout the nineteenth century. The disease manifested itself in grazing animals causing "trembles" and was transferred to humans through the animals' milk. Investigations in the late nineteenth and early twentieth century showed that "white snakeroot" (Eupatorium urticaefolium) was the cause of the illness in the Mid-west region.[51,52] When the settlers moved further west, the illness seemed to follow them. In the southwest region "trembles" was also a prevalent disease, even though no "white snakeroot" was found. Studies showed that "rayless goldenrod" (Aplopappus heterophyllus) was the cause of the malady in this region.[53] Fortunately, the disease is no longer a problem because farmers have been trained to eradicate the weed before the animals can graze on it and any contaminated milk that might enter the food chain is vastly diluted in modern processing plants.

Naturally, chemists turned toward these plants in an attempt to isolate the toxic components. Initial work on "white snakeroot" was confusing and it was not until Couch started studying the plant that any progress was made.[51] Using alcohol extraction, followed by a laborious alcohol-water separation, Couch was able to show that the

toxic component was soluble in only 30 percent alcohol-water and was isolated after base hydrolysis of this fraction. After extensive crystallizations of this "sterol" fraction, Couch reported the isolation of a crystalline sterol, mp 148 - 149° of the composition $C_{18}H_{30}O$ which was non-toxic. Also isolated was an oil which was toxic to sheep and guinea pigs. Attempts to separate the oil into other compounds failed and it was assumed pure. Couch named the oil, which he thought to be one compound, "tremetol."[51,54] Chemical studies showed it has the formula $C_{16}H_{22}O_3$, is a secondary alcohol, decomposes on distillation at 3 mm, absorbs 2 moles of Br_2 (two double bonds), has an $[\alpha]_D = -21.08°$, forms no ketone derivatives, and has a characteristic red color at the interface of a pet-ether solution of "tremetol" and concentrated sulfuric acid. Later studies by Couch showed that "tremetol" was also the toxic constituent of "rayless goldenrod."[53] Though the plants are not in the same biological family, it is interesting that they produce the same toxin. It is also interesting that while "white snakeroot" loses its toxicity on drying, "rayless goldenrod" does so only slowly.[51,53]

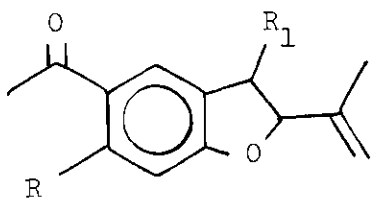
The study of these plants lay dormant for about a decade until Dermer and co-workers started a study of "rayless goldenrod."[55] Their work revealed only that "tremetol" was not a pure substance, but could be separated into other toxic fractions. No pure compound was isolated from this study.

No further work was reported in this area until the early 1960's when Bonner started his investigation of "white snakeroot"[56,57] and

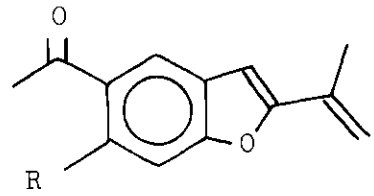
Zalkow started his study of "rayless goldenrod."[58] The similarities of compounds isolated from these plants is striking.

After extracting "white snakeroot", Bonner successfully isolated an oil which showed the characteristic color test of "tremetol."[56,57] Partition chromatography of the oil on Celite yielded a partially crystalline sterol fraction and an oily ketone fraction. Adsorption chromatography of the sterol fraction on alumina yielded a sesquiterpene-like compound ($C_{15}H_{24}$, $[\alpha]_D = +44.7^\circ$ ($CHCl_3$)), a sterol (mp 184.5 - 185.5°, $[\alpha]_D = +57.2^\circ$ ($CHCl_3$), $C_{30}H_{50}O$), and a second sterol (mp 147 - 148°, $[\alpha]_D = -32.8^\circ$ ($CHCl_3$), $C_{21}H_{34}O$). The hydrocarbon showed the presence of two nonconjugated double bonds, sterol I was similar to α-amyrin, and sterol II was similar to β-sitosterol. However, none of these compounds was toxic to goldfish and hence were not investigated further.

The ketone fraction proved to be toxic to goldfish and yielded three compounds on adsorption chromatography. These compounds were named tremetone ($C_{13}H_{14}O_2$, oil, $[\alpha]_D = -59.6^\circ$ (EtOH)), its dehydroderivative dehydrotremetone ($C_{13}H_{12}O_2$, mp 87.5 - 88.5°, $[\alpha]_D = 0.0^\circ$ (EtOH)), and a hydroxy analog hydroxytremetone ($C_{13}H_{14}O_3$, mp 70 - 71°, $[\alpha]_D = -50.7^\circ$ (EtOH)). All three ketones showed a positive "Couch" test for "tremetol." Bonner then established the structure of these compounds by degradation[56,57,60] and synthesis.[56,57,59,61,62] The parent compound, tremetone, was shown to be 2-isopropyl-2,3-dihydro-5-acetoxybenzofuran (III-1), while dehydrotremetone is unsaturated between C-2 and C-3 (III-2) and hydroxytremetone has a hydroxyl group at C-6 (III-3). Hydroxytremetone is very



III-1. R = H, R_1 = H
III-3. R = OH, R_1 = H
III-5. R = H, R_1 = OH



III-2. R = H
III-4. R = OH

similar in structure to euparin (III-4), isolated from a plant of the same family (Eupatorium pureum).[63]

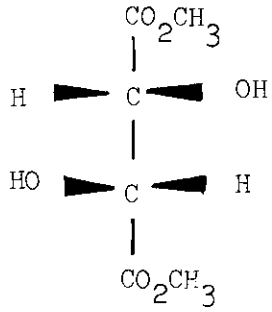
Around the same time, Zalkow and co-workers reported the results of their study of "rayless goldenrod."[58] Using both Couch's procedure (except substitution of methanol for ethanol) and modified procedures, they were able to isolate "tremetol" from the plant. Partition chromatography using Bonner's procedure gave, in the first fraction, dehydrotremetone (III-2) and a second, more polar fraction. Adsorption chromatography of "tremetol" yielded dehydrotremetone and a more polar component, toxol.[36,58] Toxol ($C_{13}H_{14}O_3$, mp 52 - 53°, $[\alpha]_D$ = -25.1°) was shown by IR and NMR to be an aromatic compound, containing one isolated double bond, a conjugated ketone, and a secondary hydroxyl group. Other experimental evidence showed toxol to have structure (III-5).

Since these investigations were conducted in order to find the toxic constituent, it appears that one should say something about the results in this area. Using sheep as a screening animal, Couch was able to show that "tremetol" caused trembles.[51-54] Couch also used

guinea pigs, cats and rabbits for his studies, but found sheep to be best.[51] All animals that died of trembles showed intense acetone concentrations in the urine and lungs (breath). Internal damage was confined mostly to the liver, kidneys, spleen and lungs. Blood sugar level was depressed. Bonner studied the toxicity of tremetone on goldfish, mice, rabbits, sheep, chickens and insects.[62] Crude "tremetol" was found to cause the death of goldfish in 10 minutes (50 mg/l dose). Dehydrotremetone (30 mg/l) caused death in 20 minutes, tremetone (30 mg/l) in 24 minutes, and hydroxytremetone (30 mg/l) in 21 minutes. Tremetone did not cause trembles in any of the test animals, but did prove toxic toward them. It was relatively ineffective as an insecticide. Zalkow found that a bacteriological screen was satisfactory for toxicity studies and, after many attempts, settled upon Bacillus cereus as the screen.[58] From this screen he was able to judge that toxol was more toxic than dehydrotremetone. However, toxol is not present in "white snakeroot" and it is unlikely that it is the cause of trembles. A study on sheep was undertaken. The crude "tremetol" that Zalkow isolated from "rayless goldenrod" did cause trembles in sheep; however, when toxol was fed to sheep, no symptoms of trembles were noted.[64] The agent that causes trembles is still unknown.

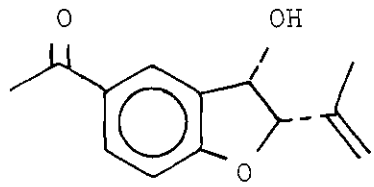
Further work was needed to fully establish the structures of the benzofurans isolated. Tremetone and hydroxytremetone both contain one asymmetric center and their absolute configurations needed to be established. Toxol contains two asymmetric centers and the absolute configuration of both centers needed to be established. When toxol was ozonized in acetic acid at room temperature for 30 hours, a keto-acid was isolated and was

then esterified with diazomethane.[36] Treatment with alkaline iodine, re-esterification with diazomethane and chromatography on silica gel gave d-dimethyltartrate (III-6). d-Dimethyltartrate could be obtained directly

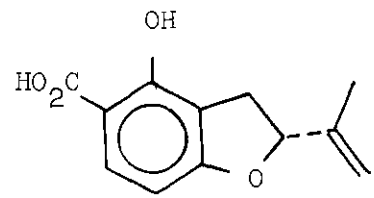


III-6

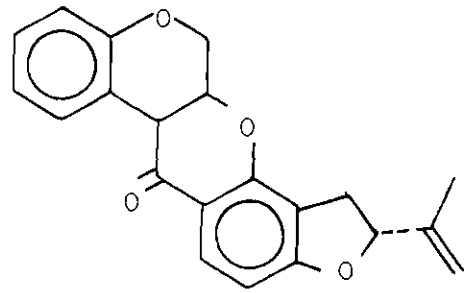
from toxol by ozonolysis in acetic acid, followed by oxidation with hydrogen peroxide and esterification. The tartaric acid isolated had a mp of 171° (reported 170°), $[\alpha]_D$ of +8.40° (water, reported +12°). The ester showed a bp of 65° at 1.2 mm and an $[\alpha]_D$ of +10.81° (MeOH, reported +13.82°). The ester showed a plain, negative ORD curve, as reported.[66] From this evidence one can only conclude that toxol has a cis relative configuration at C-2 and C-3. One can also conclude that the absolute configuration must be cis and R (III-7). Later work[65] was then done to relate the absolute configurations of tremetone and toxol to the absolute configuration of tubaic acid (III-8), a degradation product of rotenone (III-9), a potent fish toxin whose absolute configuration has been established by Büchi[67] and Nakayaki.[68] In the same study[65] tremetone was related to methyl D-(+) malate, establishing the configuration at C-2 as R. Toxol was related to (-) dihydrotremetone, clearly showing



III-7



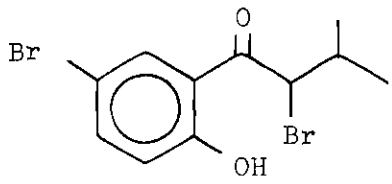
III-8



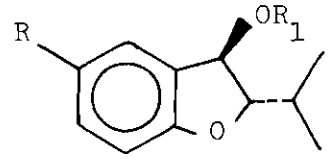
III-9

that its configuration at C-2 must be the same as in tremetone. These studies firmly established the absolute configuration of tremetone and toxol at C-2 to be R. Since toxol must have (based on just the ozonolysis experiment) a cis relationship at C-2 and C-3, the configuration at C-3 must also be R.

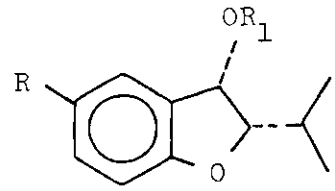
Synthesis of dihydrotremetone,[59] tremetone,[61,62] and dehydrotremetone[69] rapidly followed structure elucidation. The synthesis of dihydrotoxol was first reported by Zalkow and Ghosal in 1967[70] and later expanded upon.[37] Synthesis of 2'-hydroxy-2,5-dibromo-3-methylbutyrophenone (III-10) was readily accomplished by treatment of phenol with isovaleryl chloride, followed by a Fries rearrangement and treatment with bromine in acetic acid. Reduction of (III-10) in aqueous ethanolic KOH with sodium borohydride yielded trans-2-isopropyl-3-hydroxy-5-bromo-2,3-



III-10



III-11. R = Br, R_1 = H
III-12. R = Br, R_1 = $COCH_3$
III-13. R = CO_2H, R_1 = H
III-14. R = $COCH_3$, R_1 = H



III-15. R = Br, R_1 = H
III-16. R = Br, R_1 = $COCH_3$
III-17. R = CO_2H, R_1 = H
III-18. R = $COCH_3$, R_1 = H
III-19. R = $COCH_3$, R_1 = $COCH_3$

dihydrobenzofuran (III-11) (stereochemistry given here is as reported in the original paper). Conversion of (III-11) to trans-2-isopropyl-3-hydroxy-5-acetoxy-2,3-dihydrobenzofuran (III-14) was readily accomplished by treatment of (III-11) with butyllithium, followed by carbon dioxide to give (III-13), which was converted to (III-14) by reaction with methyllithium. The spectral properties (IR and NMR) did not match those of dihydrotoxol. Since the previous work[36] had shown toxol to be cis at C-2 and C-3, these compounds were assigned the indicated trans configuration at C-2 and C-3. A similar series of compounds could be prepared having a configuration that could be related to toxol at C-2 and C-3, as shown by direct comparison of IR and NMR spectra.

Treatment of (III-10) with sodium borohydride in ethanol with no KOH, followed by treatment with ethanolic KOH, gave cis-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (III-15). Conversion of (III-15) to (III-17) then to (III-18) was accomplished as in the trans series. Spectral properties of (III-18) were identical with those of dihydrotoxol, hence this series of compounds was assigned the indicated cis configuration.

Based on the known configuration of toxol, the assignments of the configuration of the synthetic material appeared to be firmly established. However, an odd result was observed in the NMR coupling constants between the protons on C-2 and C-3. In his study of the theoretical prediction of vicinal proton coupling constants, Karplus showed that[71]

$$J_{ab} = 4.22 - 0.5\cos\phi + 4.5\cos 2\phi$$

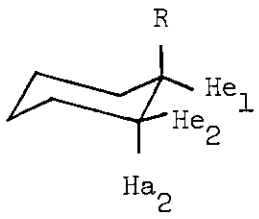
where

J_{ab} = vicinal coupling constant in Hertz

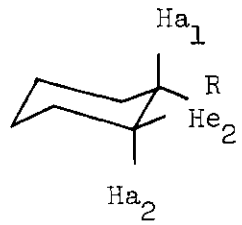
ϕ = dihedral angle between H_a and H_b.

Karplus later was one of the first to note that the above relationship has to be regarded as a zero-order approximation.[72] He noted, in particular, that electronegativity differences between a substituent and hydrogen can affect the size of the coupling constant. Booth expanded upon this prediction and established a set of statements concerning the size of vicinal proton coupling constants.[73] In

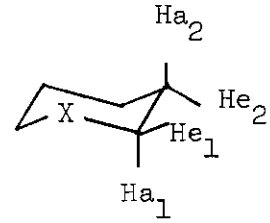
particular he stated that for an electronegative substituent on a cyclohexane ring, the maximum electronegativity effect on the coupling constant will be experienced if the substituent is axially oriented. For this case $J_{H_{e_1} H_{a_2}}$ in (III-20) is less than $J_{H_{a_1} H_{e_2}}$ in (III-21). Booth also noted that if the protons are part of a six member hetero-



III-20



III-21



III-22

cycle system (III-22) where X = N or O in a chair configuration, a maximum effect on the vicinal coupling constant will be shown by H_{e_2}. It is predicted that for this system, $J_{H_{a_1} H_{e_2}}$ will be smaller than $J_{H_{e_1} H_{a_2}}$. It is clear from both these papers that an electronegativity effect on vicinal coupling constants is a more than likely possibility.

Let us now look at the C-2 and C-3 proton vicinal coupling constants found in Zalkow's synthetic benzofurans:

Compound	$J_{2,3}$(Hz)
III-11	6
III-12	6
III-13	NA
III-14	6
III-15	4
III-16	3.5
III-17	4
III-18	4
III-19	4

Compounds (III-11) to (III-14) have been assigned a trans configuration ($\varphi \approx 100^{\circ}$) based on the fact that (III-14) was not identical spectrally with dihydrotoxol; likewise, (III-15) to (III-19) were assigned a cis configuration ($\varphi \approx 0^{\circ}$) because (III-18) was identical with dihydrotoxol. The predicted coupling constants from Karplus's equations are approximately J = 0 Hz for (III-11) to (III-14) and J=8 Hz for (III-15) to (III-19) which can conveniently be expressed as $J_{cis} > J_{trans}$. The observed coupling constants lead to the obvious conclusion that $J_{trans} > J_{cis}$, which is a reversal of the Karplus prediction. Zalkow attributed this partially to Booth's electronegativity effects. Also, in the cis isomer, the C-2 and C-3 substituents will tend to bend away from each other to relieve steric repulsions and approach a 180° angle between the C-2 and C-3 protons, which is the angle of maximum electronegativity effect and the minimum of $J_{2,3}$.

However, since the above results were reported, several reports have appeared that contradict, but do not directly disprove, these results. In particular, Tarbell has observed the correct relation of coupling constants for 2-alkyl-3-methyl-2,3-dihydrobenzofurans.[74,75] The stereochemistry of Tarbell's compounds was firmly established on the basis of equilibration of the cis isomer to what was felt to be the more stable trans isomer in sulfuric acid. These compounds have alkyl substituents at C-2 and C-3 and hence should not show the electronegativity effects predicted. Pappas and co-workers prepared several 3-hydroxy-2-phenyl-2,3-dihydrobenzofurans. Stereochemistry of the compounds was decided by the shielding

effects of the C-2 phenyl group.[76] They found that $J_{cis} > J_{trans}$ as predicted. These compounds do not have the large steric requirement of an isopropyl group at C-2, but do have the electronegativity effect. Mertes and co-workers have reported that 3-hydroxy-2-methyl-2,3-dihydrobenzofurans follow Karplus's equation.[77] The stereochemistry was confirmed by X-ray analysis. Again, these compounds have the electronegativity requirement, but no steric effect.

While the above results cast doubt on the results of Zalkow and Ghosal[37] they do not directly invalidate them. However, it was decided that the reported reversal of Karplus's equation should be confirmed. The following is a report of our findings on an X-ray study of the compound we have reported as trans-2-isopropyl-3-hydroxy-2,3-dihydrobenzofuran (III-11).

Experimental

Crystallization of cis-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran

From a bottle labelled trans-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (III-11) was taken a small amount of previously prepared material.[37] The material was dissolved in a minimum amount of 95 percent EtOH on a steam bath in a non-temperature-controlled room during the latter part of September. After the compound had dissolved the steam was turned off and the flask left on the steam bath for about 20 hours. Generally, the procedure gave crystals that were suitable for X-ray analysis. The crystals formed in this manner were thin plates. A check of the crystal batch showed that it

had a melting point, NMR, and IR identical to those reported.[37] X-ray study of these crystals showed them to belong to the space group Pbca (see below);[45] however, crystals of a triclinic space group were observed in some preparations. Attempts to reproduce the orthorhombic space group in a temperature-controlled (approximately 25°) laboratory failed, even with variations in the alcohol-water mixture and the rate of cooling.

Space Group Determination

A carefully selected crystal which showed good extinction properties under a polarizing microscope was mounted directly on a thin glass fiber using Duco Cement. The fiber was then placed on a standard goniometer head. Orientation photographs were taken on a Charles Supper Precession Camera using Cu white X-radiation and a precession angle of 10°. Orientation photographs indicated two zones at 90.0° intervals, each having mirror-mirror symmetry. Space group photographs were then taken using Cu Kα radiation (λ = 1.5418 Å). Two zero level photographs (0kl and hk0) were taken as well as the hkl upper level. The 0kl photograph showed a k(b*) spacing of 63.3 mm for four intervals or 5.8 Å spacing in the b direction of the unit cell. Similarly, the l (c*) direction showed 81.9 mm for 20 intervals or 22.6 Å spacing in the c direction, and from the hkl photo, the h(a) direction was found to be 16.6 Å (100.0 mm for 18 intervals). The unit cell volume is found to be 2200 Å^3. Later diffractometer alignment refined these figures to a = 16.692 ± 0.007 Å, b = 5.869 ± 0.003 Å, c = 22.724 ± 0.013 Å, α = 90.000 ± 0.028°, β = 90.000 ± 0.036°, γ = 90.000 ± 0.018°, Vol. = 2226.312 Å^3. The crystal was

mounted along the needle (b) axis for all studies.

The density of the crystal was found to be 1.556 gm/cm^3 by flotation in a mixed solvent system of ethyl bromide and bromotrichloromethane. Calculated density from the above data was found to be 1.554 gm/cm^3 (assuming eight molecules per unit cell).

Systematic absences were observed for 0kl: k = 2n; h0l: l = 2n; and hk0: h = 2n. This corresponds to the space group Pbca (#61).[45] The dimensions given above are for axes labelled in accord with standard procedure.

After the above crystal had decomposed, using the same crystallization procedure as before, an attempt was made to grow new crystals. A crystal was mounted from this batch and was found to be triclinic. No attempt was made to further identify the species.

A later attempt to grow the orthorhombic space group crystals in a temperature-controlled environment yielded one batch of crystals which showed six-fold symmetry. No attempt was made to study these further. Also found, from a batch grown as before from 89 percent EtOH-H_2O, was a triclinic crystal. This was studied further and showed the following dimensions: a = 12.818 Å, b = 7.844 Å, c = 11.819 Å, α = 90.75°, β = 90.02°, γ =128.02°, Vol. = 936.417 $Å^3$. The density found was 1.408 gm/cm^3. Flotation in EtOH-CCl_4 was used for this determination. From the found density, the number of molecules per unit cell was found to be three, hence the space group is probably P1.[45] No attempt was made to collect data on this crystal.

Data Collection

All data collection was performed on crystals of the space group Pbca. Zero and first layer photographs were taken of all crystals prior to data collection and cell dimensions were checked to insure that all data was collected from the same crystal type.

Initial attempts were made to collect data using film methods. The crystal was placed on a precession camera and a series of layer photographs were recorded on Ilford D X-ray Film (Industrial type) using Mo K_α radiation (λ = 0.7107 Å) and a 30° precession angle.[78] A screen was used to insure photographs of the desired layer only. Exposures were taken for 100 hrs, 10 hrs, and 1 hr. All films were developed together using Kodak Rapid X-ray Developer (3 min), a water stop bath (15 sec), and Kodak X-ray Fixer with Hardener (5 min). Washing for at least 20 min followed. The film was placed in the film cassette under an orange safe light which was at least four feet from the film at all times. All handling of film that had been exposed to X-rays was done without a safe light. The 0kl and 1kl layers were photographed. Development of the 2kl layer indicated severe decomposition had occurred. Intensities were read from the film by visually comparing the spot intensities with those of a previously prepared standard set of intensities. Because of the mirror-mirror symmetry of the photographs, only one quadrant of the film needed to be read; however, two quadrants were read to give an average intensity. These photographs yielded 63 non-zero data points ranging as follows: 0 2 0 to 0 2 18, 0 0 4 to 0 0 22, 0 4 0 to 0 4 9, 1 0 4 to 1 0 18, 1 1 1 to 1 1 10, 1 2 1 to 1 2 17, 1 3 1 to

1 3 12, and 1 4 3 to 1 4 5. However, since the crystal was decomposing during data collection and a complete data set had not been collected, no attempt was made to determine a structure from this data.

A second crystal (1.2 mm x 0.05 mm x 0.45 mm) was then mounted along the b (needle) axis on a standard goniometer head and roughly aligned on a precession camera. It was then placed on a Picker 4 Circle Diffractometer and aligned using standard procedures.[79] Alignment proved difficult, as the crystal was decomposing during the process. The positions of all possible reflections were calculated with a Burroughs B5500 Computer using a modified version of the Picker program, from the measured positions of nine reflections.[80] From this data the unit cell parameters given previously were calculated. Data was collected for θ values of 0.00° to 15.00°. A total of 589 possible reflections were calculated and punched on control cards. An additional 688 reflections were later calculated and collected. These reflections were not above background because of crystal decomposition.

The cards produced were then used to control the diffractometer for data collection. Intensity of the Zr filtered Mo radiation (λ = 0.7107 Å) was recorded for each reflection with a scintillation counter and displayed on a chart recorder. The number of counts recorded was punched on the control card and also recorded on paper tape. Calibrated Cu foil attenuators were automatically placed in front of the counter when the counts exceeded a pre-set limit

(10^4 counts/sec) and the reflection was re-collected. Data was collected in the θ - 2θ scan mode. A range of $\pm 1^{\circ}$ around the calculated peak center was scanned in 120 sec (1°/min scan rate). Background intensity was measured by fixing the counter at each end of the scan and recording counts for 20 seconds. While it is usual procedure to rescan periodically one or more reflections to get an idea of the quality of the data set, crystal alignment, and any crystal decomposition, no standard reflection was used for this crystal.

Calculation of Intensity Data

Background counts obtained at each end of the scan were averaged and multiplied by a time factor (six) to give the total number of background counts for the scan time.[81] This number was then subtracted from the total count to give the background corrected intensity. A measure of the error for each reflection was found by the following:

$$\text{ERROR} = \sqrt{RI + 0.25(BI_1+BI_2)\ T^2}$$

where RI = Raw Intensity

BI_1 = Background intensity one

BI_2 = Background intensity two

T = Time factor.

A weighting term, the above error term squared divided by the corrected intensity, was calculated for use in a later weighting scheme. All background corrected intensities that were greater than

zero were used for later data refinement and the calculation of the structure. Out of 1277 collected reflections, only 265 were non-zero. The range of non-zero reflections spanned h = 0 to 14, k = 0 to 7, and l = 0 to 16, with many gaps, especially in the k direction.

The raw, corrected intensities were then corrected for Lorentz and polarization factors. No correction was made for possible $K\alpha_1$ and $K\alpha_2$ splitting.

Calculation of the Patterson Function

Initial phasing of the structure was performed by locating the position of the heavy atom (Br) through the use of the Patterson function.[82] A Patterson function was calculated from the above 265 corrected intensities. Through the use of symmetry, the 265 terms were expanded to 857 terms for the calculation. The vector densities calculated from the Patterson function were displayed in sheets using unit cell coordinates. x ran from 0 to 0.5 in steps of 0.02, y was displayed in the same manner, and z ran from 0.0 to 1.0 in steps of 0.02. Densities displayed were normalized to 999. For the Pbca space group, the heavy atom positions are located by finding intense peaks of the Br - Br vector which are located on the Harker planes[83] at $\frac{1}{2} \pm 2x, \pm 2y, \frac{1}{2}$; $\frac{1}{2}, \frac{1}{2} \pm 2y, \pm 2z$; and $\pm 2x, \frac{1}{2}, \frac{1}{2} \pm 2z$. For $x = \frac{1}{2}$, an intense peak was found at y = 0.340 and z = 0.360, giving a z coordinate for the Br as ± 0.18 and a y coordinate of ± 0.08. At $y = \frac{1}{2}$, an intense peak was observed at x = 0.360, z = 0.860, giving an x coordinate of ±0.18 and a z coordinate of ±0.18. This gave the rough coordinates of the bromine atom as (taking the plus direction only since the presence of two mirror planes makes the plus and

minus positions the same) x = 0.18, y = 0.08, and z = 0.18. Closer study of the vector density maps showed that the x coordinate was probably closer to 0.175 and the z coordinate closer to 0.185, hence these coordinates, along with the above y value, were used for the initial phasing of the Fourier synthesis.

Location of Other Atoms, Final Refinement

The above positions of the Br were then used in a structure factor calculation. Calculation of the structure factor involves taking the following sum:

$$F_{hkl,cal} = \sum_{j}^{n} f_j e^{2\pi i(hx_j + ky_j + lz_j)}$$

where n = number of atoms in the unit cell with known coordinates

f_j = the scattering factor of the j^{th} atom.

The scattering factor is a measure of the scattering power of the spherical atom and is dependent only on the atom type and sin θ/λ. Values for the scattering factor of an atom have been calculated and tabulated.[84] Copies of these tables are introduced as data with the computer program for the calculation of the scattering factor. Scattering power is also dependent upon the thermal motion of the atom in the molecule. This effect is expressed as a thermal factor, t_f, where $t_f = \exp(-B_j \sin^2\theta/\lambda^2)$ and B_j is the thermal parameter. B_j is determined by the program using a least square procedure.

The phase of the reflection was determined in the scattering

factor program from the position of the atoms in the unit cell. Initial phases were based on the position of the Br atom alone. Introduction of the positions of other atoms in the molecule phased more reflections until the best fit was obtained between the calculated structure of the molecule and the observed structure, as measured by the R value.[85]

From the phases determined by the structure factor program and the measured intensities (corrected for background and Lorentz - polarization effects), an electron density map of the unit cell was calculated using Fourier transform techniques. It can be shown that the electron density can be expressed by the following:

$$\rho(x,y,z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} F_{hkl} e^{-2\pi i(hx + ky + lz)}$$

and for the centrosymmetric space group of this crystal:

$$\rho(x,y,z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} | F_{hkl} | \cos 2\pi(hx + ky + lz - \alpha'_{hkl})$$

where $\rho(x,y,z)$ = electron density at x,y,z

V = volume of unit cell

F_{hkl} = structure factor

$| F_{hkl} |$ = measured amplitude ($\sqrt{\text{intensity}}$)

$2\pi\alpha'_{hkl}$ = phase angle.

From this expression one can say that the electron density is the Fourier transform of the structure factor and conversely, the structure factor is the Fourier transform of the electron density. Also this expression provides the method needed to relate our measured data to the calculated structure.

An electron density map using the phases found from the Br position alone revealed the rough positions of all the other atoms in the molecule. The R factor for the calculation using Br alone was 40 percent, far below the 83 percent R factor for a random distribution of atoms in a centric space group.[85] The cis relationship of the hydroxyl to isopropyl group was apparent from a rough model. At this stage a careful study of the reflections used in the calculations was undertaken and 11 reflections which showed non-uniform background were removed from the data set leaving 254 non-zero reflections.

Introduction of the rough positions of the remaining atoms into the structure factor program immediately lowered the R value to around 25 percent. At this stage further refinement by the method of least squares was undertaken.[86] Least squares involves the minimization of the following function:

$$D = \sum_{hkl} w(|F_o| - |kF_c|)^2$$

where D = dummy variable

w = weight assigned each reflection

$|F_o|$ = observed amplitude of the structure factor

k = scaling factor

$|F_c|$ = calculated amplitude of the structure factor.

Initial least square calculations used unity for the weight assigned each reflection. Using the weighting scheme mentioned previously no improvement in the R value was observed, therefore it was decided to continue using unit weighting. We can represent the structure factor as a series expansion:

$$F = \sum_j p_j x_j$$

where x_j represents any variable of the structure factor (i.e., x,y,z positions and thermal parameters). When comparing the observed structure factor to the calculated structure factor in this minimization procedure, a Δp term arises which is representative of the parameter shift needed to fit the terms closer. This parameter shift is applied to the x, y, and z positions, the scale factor, and the thermal parameter. A new structure factor calculation is performed and a new map is calculated. The process is repeated until no further change in Δp is obtained. While, in theory, all this can be done without any operator assistance of the computer program, it has been found that the process can be enhanced by operator intervention. Application of the method of least squares led to a rapid decline of the R value to around 10.5 percent. Further application of least squares led to no significant improvement. A difference Fourier at

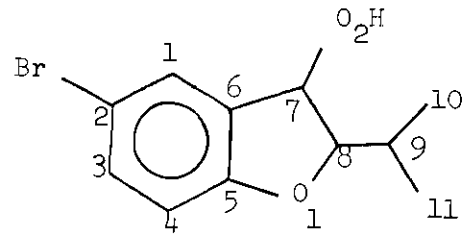
this stage was featureless, indicating that all the atoms that could be located were in proper positions.

At this stage, investigation of the electron density around the Br peak revealed that a single-parameter (isotropic) thermal factor was an inadequate description of the thermal motion of the atom. An anisotropic thermal parameter (six parameters which describe the size and shape of the thermal ellipsoid)was used. Improvement of about 0.75 percent was noted giving a final R value of 9.74 percent. No correction was made for absorption effects as it was felt the data was not good enough to warrant this correction.* For each of the 13 non-bromine atoms located, there were three position parameters and one thermal parameter and for the Br atom there were three position parameters and six thermal parameters. Hence the value of 52 variables (including a scale factor) had to be determined from just 254 data points. The fact that the initial R value with all atoms included was around 25 percent indicates that the structure is probably correct, excepting small position changes and changes in thermal parameters.[85] The low R value of the final refinement was another indication that the structure was correct.

---

* An estimation of the magnitude of the absorbance correction can be found by using the formula $I = I_o \exp(-\mu t)$, where I is the corrected intensity, I_o the observed intensity, t the thickness through which the X-rays pass, and $\mu = \rho(P_C M_C + P_O M_O + P_{Br} M_{Br})$ where ρ is the density, P is the weight fraction of the indicated atom, and M is the mass absorption coefficient of the indicated atom as tabulated in reference 84. $\mu = 38.29\ cm^{-1}$ for this crystal. The estimated transmittance factor for this crystal was 0.8 for the 0.05 mm thickness and 0.2 for the 0.45 mm thickness. Though the latter correction appears to be quite large, an error of no more than 10-15 percent was observed during the initial alignment procedures.

The final coordinates and thermal parameters are shown below:



Atom	x	y	z	B
Br	0.17731	0.07869	0.18421	*
C1	0.15880	0.06914	0.06433	2.32023
C2	0.14811	-0.03724	0.11492	0.76976
C3	0.11919	-0.26477	0.10999	3.77632
C4	0.09116	-0.34195	0.05514	3.35871
C5	0.09826	-0.22731	0.00203	2.01612
C6	0.14000	-0.00640	0.01325	2.65265
C7	0.13433	0.04566	-0.04560	8.58661
C8	0.08886	-0.10689	-0.09455	1.77152
C9	0.10489	-0.17843	-0.15006	4.97118
C10	0.05701	-0.34816	-0.17840	5.64249
C11	0.11866	0.04741	-0.19347	9.09483
O1	0.08223	-0.28114	-0.05157	4.06910
O2	0.21711	0.07741	-0.07495	7.24093

* Anisotropic thermal parameters for Br were: B_{11} = 0.00447, B_{22} = 0.06092, B_{33} = 0.00562, B_{12} = -0.00058, B_{13} = 0.00024, B_{23} = -0.00309.

The final calculated structure factors versus the observed structure factors are shown on the following page.

Calculation of Molecular Data

Bond angles and bond lengths were calculated from the above data in the standard manner and are shown below:

Table 3. Calculated Structure Factors Versus Observed Structure Factors for (III-11). R = 0.0974.

L= 0

H	K	FO	FC
2	0	140	127
4	0	350	347
6	0	86	75
8	0	96	97
12	0	76	84
14	0	34	47
2	1	112	102
4	1	275	261
6	1	91	83
8	1	131	113
10	1	31	25
0	2	106	112
2	2	143	143
6	2	162	167
8	2	27	30
10	2	48	49
2	3	155	155
4	3	111	107
6	3	33	22
10	3	42	50

L= 1

H	K	FO	FC
1	1	154	139
2	1	108	119
3	1	239	226
4	1	181	178
5	1	67	51
6	1	91	71
7	1	71	55
8	1	107	107
9	1	44	30
10	1	111	103
0	2	95	124
1	2	106	105
2	2	83	73
4	2	55	53
5	2	21	25
6	2	91	86
7	2	57	51
8	2	72	67
9	2	27	19
10	2	34	28
14	2	30	48
1	3	47	47
0	4	38	40
1	4	26	29
2	4	28	25
3	4	25	26
5	4	26	23
6	4	28	40
8	4	33	50
4	5	33	24

L= 2

H	K	FO	FC
0	0	31	55
1	0	124	126
2	0	89	83
3	0	105	84
4	0	80	72
5	0	222	211
6	0	179	152
7	0	172	164
8	0	107	102
9	0	49	2
1	1	72	76
2	1	125	130
3	1	38	39
4	1	15	10
5	1	27	20
8	1	40	32
9	1	68	61
10	1	63	54
11	1	48	54
0	2	57	69
1	2	54	62
4	2	28	23
5	2	55	46
7	2	85	72
8	2	59	51
10	2	40	46
1	3	40	37
2	3	23	17
3	3	75	69
4	3	73	69
5	3	43	39
6	3	25	21
8	3	28	36
0	4	29	43

L= 3

H	K	FO	FC
1	1	79	77
2	1	61	54
3	1	173	158
5	1	91	88
6	1	55	58
8	1	44	52
9	1	62	65
11	1	88	94
1	2	99	94
2	2	62	67
3	2	28	33
5	2	52	54
6	2	48	48
7	2	110	110
9	2	38	34
1	4	52	43
2	4	29	23

L= 4

H	K	FO	FC
0	0	52	63
1	0	190	192
2	0	22	23
3	0	141	124
5	0	70	60
7	0	143	143
9	0	90	107
1	1	72	61
2	1	23	21
3	1	102	98
5	1	67	69
6	1	30	29
1	2	75	77
3	2	48	40
5	2	29	29
7	2	50	52
8	2	27	23
1	3	47	49
3	3	104	87
4	3	23	26
5	3	80	78
9	3	27	45

L= 5

H	K	FO	FC
1	1	54	52
2	1	76	76
3	1	146	136
4	1	57	65
5	1	65	66
6	1	49	42
7	1	29	36
9	1	51	53
10	1	26	27
11	1	57	58
0	2	52	64
1	2	92	95
2	2	59	56
5	2	52	52
6	2	30	33
7	2	78	73
9	2	30	38
1	4	50	52

L= 6

H	K	FO	FC
0	0	116	123
1	0	44	50
2	0	35	35
3	0	67	62
4	0	61	60
5	0	62	53
6	0	122	107
7	0	106	93
8	0	68	66
9	0	59	58
1	1	38	42
2	1	31	37
3	1	34	44
4	1	45	51
9	1	38	39
10	1	45	42
0	2	20	27
1	2	40	43
2	2	25	22
6	2	42	47
7	2	39	37
8	2	25	20
9	2	30	34
2	3	65	59
3	3	59	51
4	3	47	33
5	3	65	67

L= 7

H	K	FO	FC
2	1	86	91
3	1	57	56
4	1	97	94
5	1	60	61
6	1	77	74
10	1	68	83
0	2	70	73
1	2	66	82
2	2	87	89
3	2	24	15
5	2	23	21
6	2	90	100
8	2	53	49
1	3	28	28

L= 8

H	K	FO	FC
0	0	224	239
2	0	91	89
3	0	43	45
6	0	92	74
8	0	132	130
1	1	32	40
2	1	82	74
3	1	26	30
4	1	106	107
0	2	65	73
1	2	22	28
2	2	51	55
3	2	24	28
6	2	30	42
2	3	82	83
4	3	91	90
5	3	32	40
6	3	26	28

L= 9

H	K	FO	FC
2	1	41	43
3	1	62	57
4	1	84	74
5	1	68	70
8	1	41	48
0	2	53	56
1	2	88	98
2	2	29	31
5	2	28	26
6	2	48	55
7	2	34	32
8	2	41	40

L=10

H	K	FO	FC
1	0	31	27
2	0	44	47
3	0	44	42
4	0	30	29
5	0	65	63
6	0	59	58
7	0	94	86
9	0	47	42
1	1	38	51
3	1	40	49
6	1	24	20
0	2	61	60
3	3	53	54

L=11

H	K	FO	FC
1	1	31	54
2	1	35	33
3	1	81	86
4	1	27	31
5	1	38	35
1	2	40	46
3	2	32	39
5	2	35	32
7	2	52	65

L=12

H	K	FO	FC
0	0	43	30
1	0	103	104
5	0	64	67
7	0	47	43
5	1	41	43

L=13

H	K	FO	FC
2	1	31	34
3	1	44	49
4	1	32	30
5	1	34	26
0	2	29	25
1	2	27	28
2	2	25	18

L=14

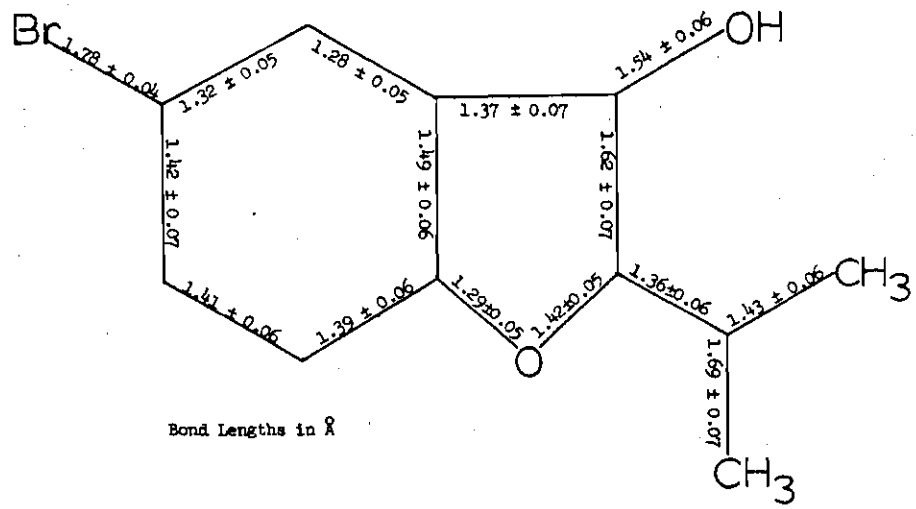
H	K	FO	FC
0	0	62	91
1	0	33	37
6	0	46	43

L=15

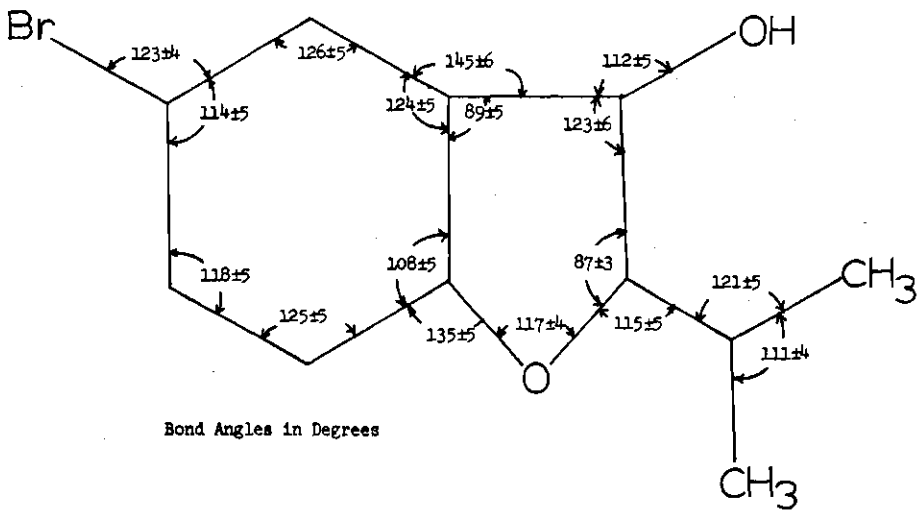
H	K	FO	FC
2	1	29	40
4	1	35	38

L=16

H	K	FO	FC
0	0	83	84
1	0	32	22
2	0	31	27



Bond Lengths in Å



Bond Angles in Degrees

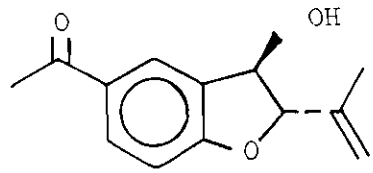
A least square plane through the atoms of the benzofuran ring was calculated. The hydroxyl oxygen was located 1.33 Å above the plane and the central carbon atom of the isopropyl group was above the plane by 0.49 Å. Inclusion of the bromine position in the calculation of a new least square plane did not affect the above results. A least square plane calculated from just the benzene ring

position showed the hydroxyl oxygen at 1.54 Å above the plane and the isopropyl carbon at 0.36 Å in the same direction.

Drawings of the molecule were made using the ORTEP program.[87] Due to the large errors observed in the thermal parameters, the atoms are shown as small balls instead of the usual thermal ellipsoids. The cis relationship is clearly evident. These drawings are shown on the following page.

Results and Discussion

It is clear from the results of the X-ray analysis that the compound that we had assumed to be trans is in reality cis. The implications of this result are twofold. First, this compound was not related to toxol[37] and we had thought toxol was cis.[36] Therefore, the synthetic series related to toxol must be trans. Toxol must be trans and since the configuration at C-2 has been fixed by comparison with rotenone and other compounds,[65] toxol must have the absolute configuration shown in (III-23). The second implication



III-23

is that the benzofurans do not show a pronounced electronegativity effect and do obey Karplus's equation ($J_{trans} > J_{cis}$).[71-73]

Our results, as presented in the previous section, are not what is generally accepted as the ultimate in X-ray structure

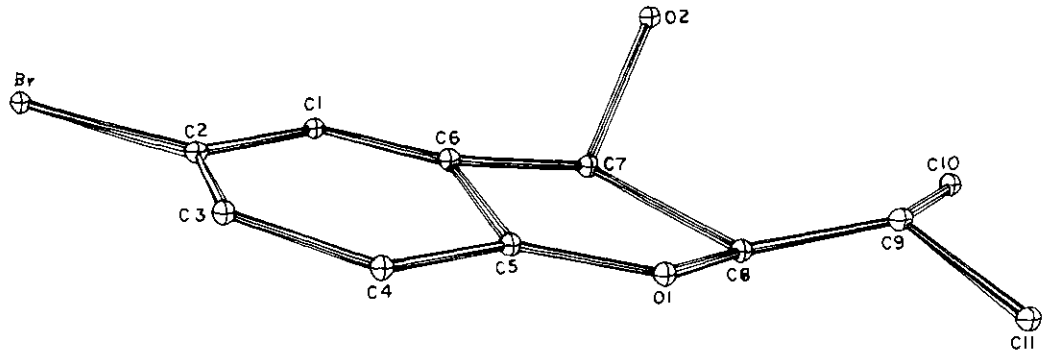


Figure 1. Drawing of (III-11) Looking Over the Benzofuran Ring.

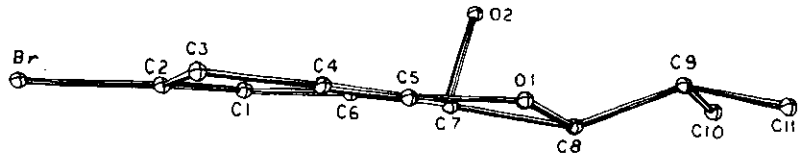


Figure 2. Drawing of (III-11) Looking into the Plane of the Benzofuran Ring. (The cis relationship of O2 to C9 is clearly evident.)

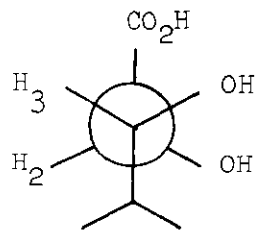
analysis. It is generally assumed that one needs about 50 reflections per atom for a reasonably accurate structure determination[88] and for our 14 atom molecule this would be 700 reflections. We have collected only 254 non-zero reflections for this crystal, approximately one-third the number for a good data set. This data was collected on a decomposing crystal and few of the reflection scans showed what is considered an ideal shape.

However, it is believed the structure is a correct one, though one should not put any reliance on the calculated bond parameters, except as general guides. In refining the data, the cis relationship of O-2 to the isopropyl group was evident from the first electron density map. It is possible that one could use least squares to fit an incorrect structure with the data set obtained, but our cis relationship was evident without any least squares calculations. Least squares did not move the hydroxyl and isopropyl groups very much, hence these atoms were located initially almost as well as possible. A difference Fourier showed no new atom positions, hence it was unlikely that O-2 and C-9 (X-ray numbering) were in false minima. Finally, the benzofuran ring is very clearly defined indicating that since the data was good enough to resolve this structure, it should be good enough to answer the simple question of cis or trans isomers.

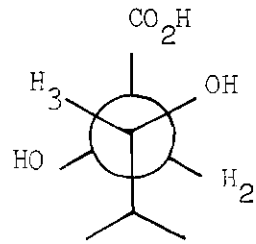
Naturally, we realize that the above arguments are all subjective and it would be better to have an objective answer to the question. The original crystal was grown in a room where the temper-

ature varied from 19° to 27° during the 24 hours it took to grow the crystals. When attempts were made to grow crystals in a temperature controlled room (25° - 27°) the space group of the previous crystal was not obtained. Whether this is due to the alcohol-water concentration used in crystallization or the variation in cooling rate is unknown; however, it is felt that the latter is the predominant reason.

Even though we felt that our X-ray structure was correct, it was decided that an independent chemical proof of the structure was in order. E. Keinan, working at the University of the Negev, Beer-Sheva, Israel, synthesized both racemic threo-2,3-dihydroxy-4-methylpentanoic acid (III-24) and racemic erythro-2,3-dihydroxy-4-methylpentanoic acid (III-25).[89,90]



III-24



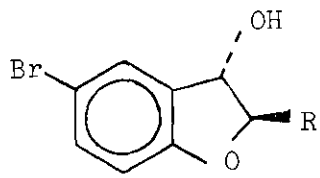
III-25

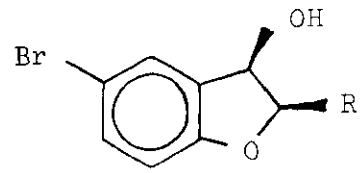
The NMR spectrum of (III-24) showed a $J_{2,3}$ of 2 Hertz and the spectrum of (III-25) showed a $J_{2,3}$ of 5.5 Hertz. Ozonolysis of the compound used for the X-ray study (independently synthesized under Dr. L. H. Zalkow's direction by E. Keinan) yielded (III-24) as indicated by the NMR spectrum of the crude reaction mixture. The

threo isomer could only arise if the compound was cis, as indicated by the X-ray study. The trans benzofuran was also synthesized and showed, as expected, identical properties to the one reported as cis. It was subjected to ozonolysis and yielded the erythro pentanoic acid, clearly indicating the trans stereochemistry. Since this compound yielded dihydrotoxol upon further reaction, it is now firmly established, by both X-ray analysis and chemical means, that toxol is trans at C-2 and C-3 (III-23).

To insure that no violation of the Karplus equation[71] exists in hydroxyalkyl benzofurans, Keinan synthesized compounds (III-26) through (III-28) using the sequence of Zalkow and Ghosal.[37] In all cases Karplus's equation was followed: $J_{2,3}$ for the trans series was 3.5 to 4.0 Hertz and $J_{2,3}$ for the cis series was 5.5 to 6.5 Hertz.

Recently, the coupling between H-2 and H-3 in toxol itself has been measured by Keinan using a Varian 100 MHz NMR on approximately



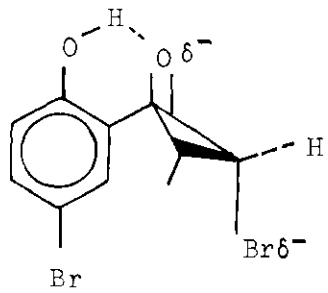


III-26. a) R = CH_3 b) R = CH_3
III-27. a) R = C_2H_5 b) R = C_2H_5
III-28. a) R = nC_3H_7 b) R = nC_3H_7

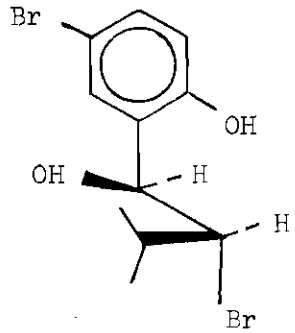
10 mg of pure toxol. The coupling was 3.7 Hertz, clearly indicating the trans stereochemistry.[89] A reproduction of the δ4.8 to 5.5

region of this spectrum is shown on the following page.

It now appears necessary to explain why one gets the cis series when base is used and the trans series when base is not used. If one applies Cram's rule[91] to the system, we see that the steric size of a Br atom versus an isopropyl group must be determined. These groups are very close in size and any strong argument for the stereochemical control should not invoke a steric argument. However, there is a great difference in the electronic requirement of Br and isopropyl. Since we have gone from a neutral medium to a basic medium to achieve the stereochemical reversal, it appears that an electronic effect is an appropriate explanation. One can assume that in non-basic medium the carbonyl of (III-10) is hydrogen-bonded to the phenolic hydroxyl. In this configuration the Br would orient itself so that its negative dipole is pointed away from the negative dipole of the carbonyl (III-29, only one of the racemates is shown). Attack by hydride would be from the backside



III-29



III-30

yielding threo alcohol (III-30) which cyclizes to the trans isomer,

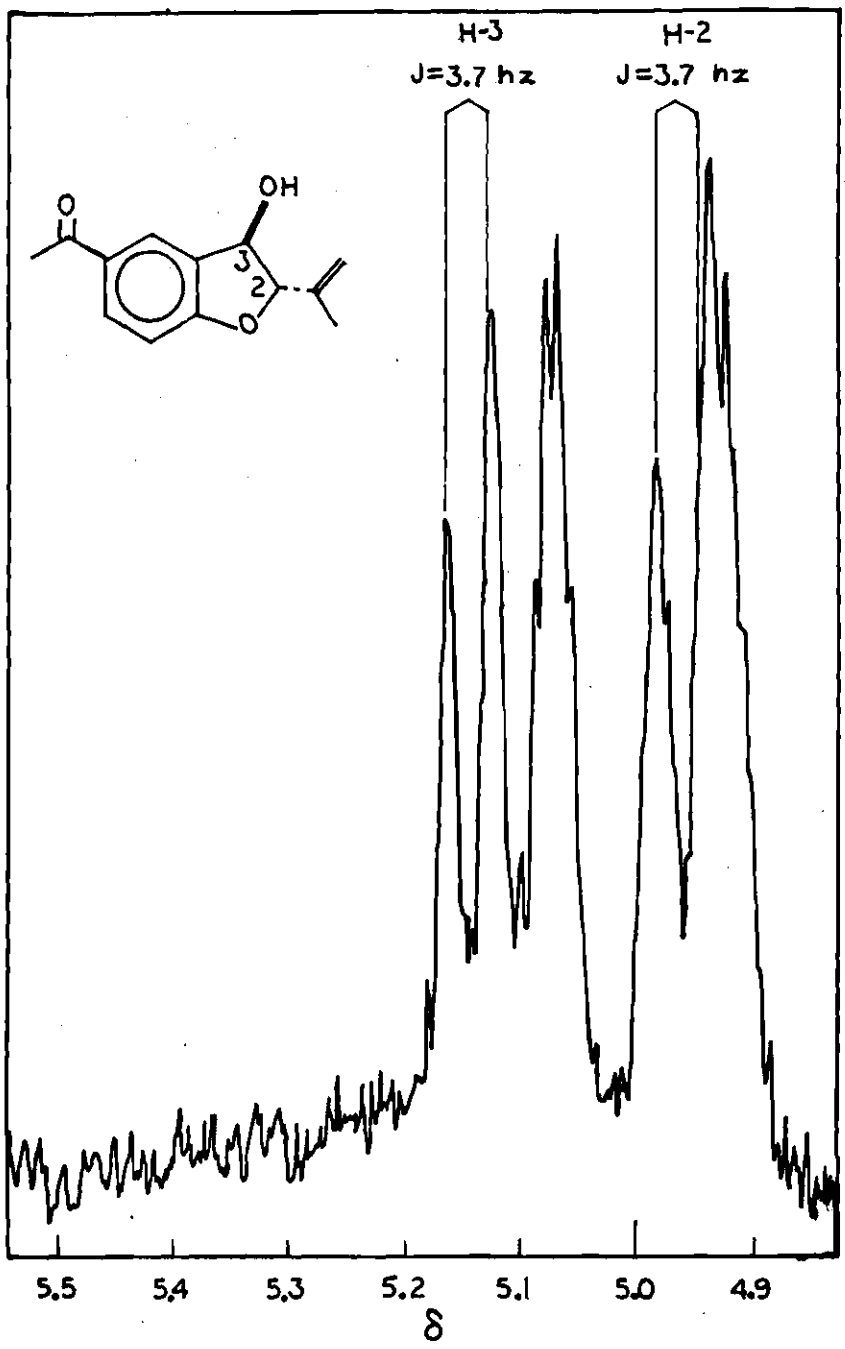
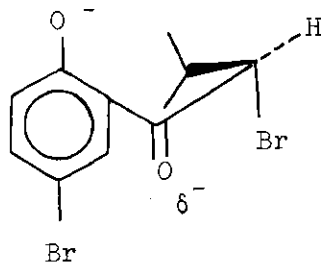
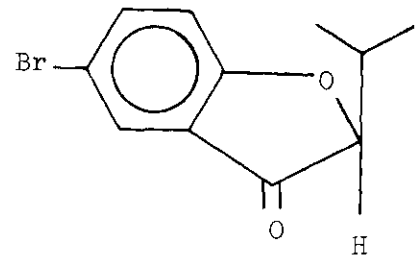


Figure 3. Reproduction of the δ4.8 to 5.5 Region of the NMR Spectrum of Toxol Showing $J_{2,3}$ = 3.7 Hz.

as shown experimentally. For the case where base is used in the reductive step the reasoning is simpler. The phenolate anion closes the furan ring by an SN-2 attack on the bromine prior to reduction and is greatly facilitated by the carbonyl dipole orienting itself opposite to that of (III-29). The bromine is now oriented for



III-31



III-32

facile SN-2 displacement (III-31) to form benzofuran (III-32). Hydride adds opposite to the isopropyl group and the cis isomer is formed. It appears that this reaction is not as stereospecific as one might expect. Keinan reports a 1:1 ratio of cis to trans isomers under these conditions. Apparently hydride attack is possible from either side.

Now that it has been established that toxol is trans, one must explain why the tartaric acid isolated showed the properties of the isomer that could only arise from the cis isomer. Unfortunately, at this time the answer is unknown. Sufficient quantities of natural toxol were not available for further work. Our only explanation at this moment is a poor one. We feel that the tartaric

acid produced was meso (the one expected from the trans configuration) and that the rotation was due to a contaminant. It should be mentioned that our observed properties of the tartaric acid were similar to, but did not exactly reproduce, those of the literature. Work is now under way at Georgia Tech on isolating sufficient quantities of toxol to redo the ozonolysis and check on product composition by NMR. This work is under reinvestigation.

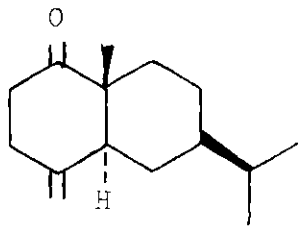
CHAPTER IV

BY SYNTHESIS: THE STRUCTURE OF CANARONE

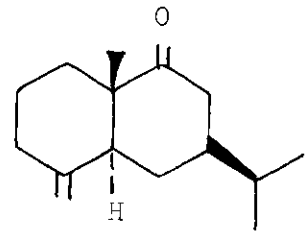
Introduction

Canarone, a monoethynoid sesquiterpene ketone, has been isolated by Bhattacharyya and co-workers from Black dammar resin (Canarium strictium Roxb.).[24] Infrared spectra showed a carbonyl on a six-membered ring and a terminal methylene group. UV showed the ketone was non-conjugated. Lithium aluminum hydride reduction gave a mono-alcohol, canarol, which gave eudalene on selenium dehydrogenation. Hydrogenation indicated only one double bond. Ozonolysis of canarol gave formaldehyde, adding weight to the IR evidence of a terminal methylene group. The non-volatile component from ozonolysis gave a negative iodoform test, indicating the methylene group was 4(14) and not on the isopropyl side chain. The position of the ketone was decided by reacting canarone with methyl magnesium iodide. After reaction with methyl magnesium iodide, and workup in the usual manner, the reaction mixture was dehydrated over selenium and filtered through alumina. An ultraviolet spectrum indicated 90 percent naphthalenic products, which was shown to be 1,4-dimethyl-7-isopropylnaphthalene by its TNB derivative, mp 102-103°, lit mp 103-104°,[92] and picrate, mp 112-113°, lit mp 113-114°.[93] From the ORD curve of canarone, Bhattacharyya concluded that the ring fusion was trans and the same absolute configuration as the eudesmane group. The isopropyl group

was given its usual equatorial orientation. From this information the structure of canarone was deduced to be as shown in (IV-1). In a later paper, with no new evidence, Bhattacharyya changed the structure of canarone to (IV-2).[94]

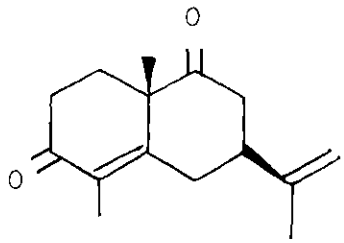


IV-1

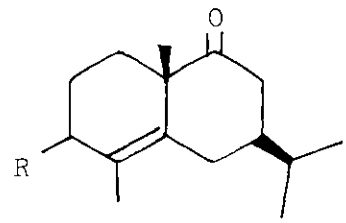


IV-2

In order to confirm the new structure of canarone, Zalkow and Lacoume synthesized (IV-2).[95] Robinson annelation of hydroxycarvone with ethyl vinyl ketone (EVK) gave, after dehydration, diketone (IV-3),



IV-3



IV-4. R = =O
IV-5. R = H_2

which was hydrogenated using Pt/C as a catalyst to give (IV-4). Thioketalization followed by desulfurization over Raney nickel gave monoketone (IV-5). Conversion of (IV-5) to (IV-2) was readily accomplished by hydroboration, oxidation with hydrogen peroxide and sodium

dichromate, and finally dehydration with $POCl_3$ in pyridine. Synthetic (IV-2) was different in both NMR and IR from canarone.

Since Bhattacharyya's revised structure of canarone has been shown to be incorrect, it was decided to synthesize (IV-1), as its enantiomer, by an unambiguous route. Comparison of NMR, IR and ORD with those of natural canarone should show whether that structure is correct.

Experimental

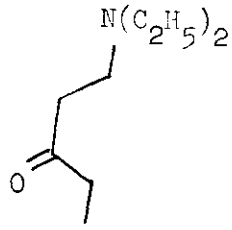
Instrumentation Used

All NMR spectra were recorded on a Varian A-60D usually using $CDCl_3$ as a solvent and TMS as an internal standard, and are reported in δ (ppm) units. IR spectra were recorded on a Perkin-Elmer 237B or a Perkin-Elmer 257 as thin, neat films. Mass spectra were recorded on a Varian M-66 mass spectrometer. Exact mass determinations were performed on a Hitachi (Perkin-Elmer) RMU-7L. UV, ORD and CD spectra were recorded on JASCO ORD/UV 5 Optical Rotatory Dispersion Recorder. Gas chromatographs were taken on either an F and M Model 400 gas chromatograph or a Hewlett-Packard Model 402 gas chromatograph using flame ionization detectors. The following GC columns were used:

Column Number	Liquid Phase	Support	Column Size
I	3 percent SE 30	100/120 Gas Chrom Q	6' x $\frac{1}{4}$"
II	3 percent QF 1	100/120 Gas Chrom Q	6' x $\frac{1}{4}$"
III	5 percent Carbowax W	90/100 AS	6' x $\frac{1}{4}$"
IV	3 percent UCON LB-550-X	100/120 Gas Chrom Q	4' x $\frac{1}{4}$"
V	10 percent XE 60	60/80 Gas Chrom Q	6' x $\frac{1}{4}$"

All columns were glass and bent into a U-shape. Helium, flowing at 88 ml/min, was the carrier gas. Elemental analyses were performed by Bernhardt Microanalysis Laboratories, West Germany; or Atlantic Microlab, Atlanta, Georgia.

1-Diethylamino-3-pentanone[97]

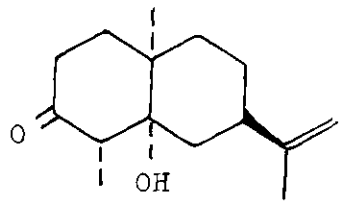


Dry ethylene gas was bubbled through a stirred mixture of 187.5 gms of propionyl chloride and 288 gms of anhydrous $AlCl_3$ in 1.25 liters of dry $CHCl_3$ at zero degrees for 10 hours. The reaction mixture was then poured onto crushed ice containing 250 ml of concentrated HCl. A white paste was immediately formed. The organic layer was washed with water, dried over $MgSO_4$ and concentrated in vacuo to approximately one liter. The $CHCl_3$ was then cooled to zero degrees and 250 gms of Et_2NH was added dropwise. The yellow reaction mixture was stirred at

zero degrees for 17 hours after which time the $CHCl_3$ was extracted with 10 percent HCl until the aqueous layer was strongly acidic. Then the aqueous layer was neutralized with 20 percent NaOH and extracted with Et_2O. The ether was dried over $MgSO_4$ and removed *in vacuo* to yield 164 gms of a crude yellow liquid. Distillation on a 36 inch stainless steel spinning band column yielded 140 gms (44 percent) of pure amino-pentanone, bp 60° at 3 mm (reported 80° at 10 mm).[96]

4aβ-Hydroxy-4α-methyl-6α-isopropenyl-8aβ-methyl-1,3,7,8-tetrahydro-naphthalen-3(2H)one[97]

To 100 gms of the above amino-pentanone was slowly added 91 gms of methyl iodide. Meanwhile, 122 gms of d-dihydrocarvone (obtained from Glidden Co.) was added to 770 ml of anhydrous ether under an N_2 atmosphere at zero degrees. To the stirred solution of d-dihydrocarvone was added 35 gms of $NaNH_2$. After one hour of stirring, the above quaternary amine salt, dissolved in 200 ml of dry pyridine, was quickly added to the stirred enolate. The reaction was stirred at zero degrees for 18 hours after which it was refluxed for five hours. The reaction mixture was cooled to room temperature, poured into 1.5 liters of cold water and extracted with ether. The ether was dried over $MgSO_4$ and removed *in vacuo* to yield 250 gms of a crude reddish oil which was distilled on a 36 inch stainless steel spinning band column. Yield was 69.8 gms of a mixture of the desired alcohol and its dehydrated analogs. The mixture was used in the next

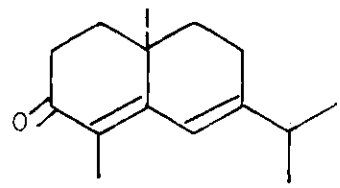


reaction.

Bp: 84-92° at 0.15 mm (reported for ketol: 140-150° at 0.5 mm).[97]

4-Methyl-6-isopropyl-8aβ-methyl-1,7,8-trihydronaphthalen-3(2H)one ((-)β-cyperone)[97]

Dehydration of the above octalone (19.52 gms) was accomplished by stirring it in 50 percent H_2SO_4 (200 ml) for 5 hours. Neutralization of the acid with solid $NaHCO_3$ was followed by ether extraction(after filtration of the inorganic salt that precipitated). Removal of the ether, which had been dried over $MgSO_4$, in vacuo, followed by vacuum distillation left 17.78 gms (99 percent) of (-)β-cyperone.



Bp: 92° at 0.075 mm (reported: 90-95° at 0.1 mm).[97]

R_t (Col. I, temp = 160°): 3.6 min.

IR (found): ν_{neat} (cm^{-1}): 1660, 1640, 1575, 1370, 1350.

IR (reported):[98] ν_{neat} (cm^{-1}): 1665, 1640, 1600, 1385, 1370.

NMR (δ, $CDCl_3$): 1.10 (6H, d, J = 7 Hz, isopropyl methyl), 1.10 (3H, s, angular methyl), 1.83 (3H, s, C-4 methyl), 6.32 (1H, bs, $w_{\frac{1}{2}}$ = 4 Hz, olefinic H).

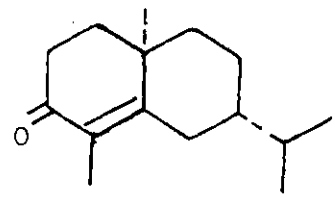
Mass Spectrum: M^+_{found} = 218 (79 percent), calculated = 218 ($C_{15}H_{22}O$) base peak (m/e) = 45.

$[\alpha]_{546}$ (found, c = 1.45, $CHCl_3$): -386°.

$[\alpha]_{546}$ (reported for enantiomer, c = 1.44, $CHCl_3$):[97] +672°.

4-Methyl-6β-isopropyl-8aβ-methyl-1,5,7,8-tetrahydronaphthalen-3(2H)one ((-) dihydro-α-cyperone)

To 500 ml of liquid NH_3 was added 18 gms of Li. After all the Li had dissolved, 17.78 gms of (-)β-cyperone, dissolved in 75 ml of absolute EtOH and 80 ml of absolute Et_2O, was added dropwise over 15 minutes. Stirring for two hours was followed by the addition of 250 ml of absolute EtOH which caused the slow disappearance of the blue color. The ammonia was allowed to evaporate overnight. Then 50 ml of water was added followed by 200 ml of 6N HCl, which reduced the pH to 8. Extraction of the aqueous layer with ether followed. The ether was dried over $MgSO_4$, removed in vacuo, and the residue was vacuum distilled to yield 14.06 gms (78 percent) of (-) dihydro-α-cyperone.



Bp: 85° at 0.05 mm (reported: 148-149° at 3.5 mm).[99]

R_t (Col. I, temp = 160°): 2.7 min.

IR (found): $\nu_{neat}(cm^{-1})$: 1660.

IR (reported):[99] $\nu_{CCl_4}(cm^{-1})$: 1665.

NMR (found, δ, $CDCl_3$): 0.93 (6H, d, J = 6 Hz, isopropyl methyl), 1.20 (3H, s, angular methyl), 1.77 (3H, s, C-4 methyl).

NMR (reported, δ, CCl_4):[99] 0.93 (6H, d, J = 6 Hz, isopropyl methyl), 1.18 (3H, s, angular methyl), 1.70 (3H, s, C-4 methyl).

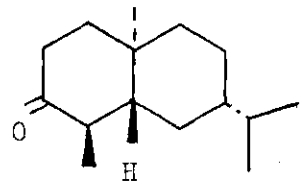
Mass Spectrum: M^+_{found} = 220 (base peak), calculated = 220 ($C_{15}H_{24}O$).

$[\alpha]_{546}$ (found, c = 1.47, $CHCl_3$): -85°.

$[\alpha]_{546}$ (reported for enantiomer, c = 3.4, $CHCl_3$):[100] +152°.

4α-Methyl-6β-isopropyl-8aβ-methyl-1,4aα,5,7,8-pentahydronaphthalen-3(2H)one

A solution of 5.0 gms of Li in one liter of liquid NH_3 was prepared. To this was then added dropwise over 20 minutes a solution of 14.06 gms of (-) dihydro-α-cyperone in 100 ml of absolute ether. The



reaction was stirred for two hours after which 34 gms of NH_4Cl was slowly added. The blue color disappeared after 15 minutes, when an additional 7 gms of NH_4Cl was added. Workup of the reaction mixture was performed as in the previous reaction to yield 12.19 gms (86 percent).

Bp: 110° at 1.2 mm (reported: 90° at 0.1 mm).[100]

R_t (Col. I, temp = 160°): 2.2 min.

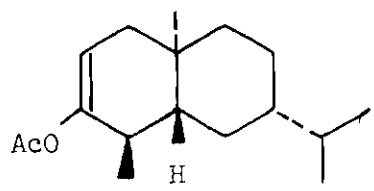
IR: $\nu_{neat}(cm^{-1})$: 1710, 1450, 1375.

NMR (δ, $CDCl_3$): 0.88 (6H, d, J = 6 Hz, isopropyl methyl), 0.98 (3H, d, J = 6 Hz, C-4 methyl), 1.08 (3H, s, angular methyl).

Mass Spectrum: M^+_{found} = 222 (41 percent), calculated = 222 ($C_{15}H_{26}O$); base peak (m/e) = 81.

3-Acetoxy-4β-methyl-6β-isopropyl-8aβ-methyl-1,4aβ,5,7,8-pentahydronaphthalene

The above saturated ketone (16.7 gms) was dissolved in 190 ml of isopropenyl acetate to which 1.5 gms of p-toluenesulfonic acid (PTSA) was added as a catalyst. The



reaction was vigorously refluxed under a Dean Stark trap. After 5.5 hours, 25 ml of liquid was removed from the trap and 25 ml of isopropenyl acetate was added to the refluxing liquid. This was repeated after 9 hours, when 30 ml of liquid was removed and 30 ml added. The reaction was allowed to continue until a total reaction time of 24 hours had elapsed. After cooling to room temperature, the reaction mixture was extracted with $NaHCO_3$ (5 percent), dried over anhydrous sodium sulfate and the solvent removed in vacuo. A VPC analysis (Col. I, temp = 160°) indicated two peaks, R_t = 2.8 min and 3.2 min, in a 2:3 ratio. NMR analysis indicated both the Δ2 and Δ3 double bond isomers.

NMR (δ,$CDCl_3$): 0.90 (6H, d, J=6 Hz, isopropyl methyl), 0.93 (3H, s, angular methyl), 1.00 (~1.8H, d, J=6 Hz, C-4 methyl for Δ2 enol acetate), 2.03 (3H, s, acetate methyl), 2.10 (~1.2H, s, C-4 methyl for Δ3 enol acetate).

The enol acetate mixture was then hydrolyzed by stirring it in a 10 percent solution of concentrated HCl in MeOH overnight. Negligible hydrolysis was observed while stirring in MeOH to which PTSA had been added. The HCl was neutralized with solid $NaHCO_3$. The MeOH was removed in vacuo and the residue was taken up in ether. Washing of the ether layer with water was followed by drying over $MgSO_4$ and removal in vacuo to give 16.1 gms of starting ketone.

A second reaction was run using the above conditions, but VPC samples were obtained at one hour, 3.5 hours, and every 30 minutes thereafter until 5.5 hours had elapsed. The ratio of

products was obtained by comparing the peak heights of the ketone, the Δ2, and Δ3 enol acetate. The results are shown in the discussion section. After 24 hours a product composition identical to the one obtained above was realized.

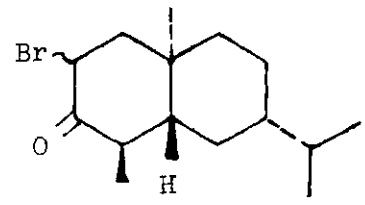
The double bond isomers could not be separated by column chromatography on basic alumina or 10 percent $AgNO_3$ - silica gel. Neither could they be separated on a spinning band column or by preparative VPC.

However, preparation of the desired Δ2 compound could be accomplished by a slight modification of the reaction conditions. To 140 ml of isopropenyl acetate was added 11.7 gms of saturated ketone and 0.75 gms of PTSA. Heating of the reaction was accomplished by using an oil bath set at 110° instead of a heating mantle, to insure a slow, gentle reflux. Workup in the previous manner yielded 13.6 gms of a 3:1 mixture of Δ2 and Δ3 enol acetates. Approximately 10 percent unreacted ketone was left. This mixture was then used without further purification.

IR: $\nu_{neat}(cm^{-1})$: 1760 (s), 1730 (s), 1675 (m), 1220 (s).

NMR (δ, $CDCl_3$): 0.90 (6H, d, J = 6 Hz, isopropyl methyl), 0.93 (3H, s, angular methyl), 0.98 (3H, d, J = 5 Hz, C-4 methyl), 2.12 (3H, s, acetate methyl).

2-Bromo-4α-methyl-6β-isopropyl-8aβ-methyl-1,4aα,5,7,8-pentahydro-naphthalen-3-one



The crude enol acetate (above, 13.6 gms) was dissolved in 210 ml of CCl_4 to which 21 ml of epichlorohydrin

was added. To this constantly stirred solution was added dropwise over 15 minutes 8.9 gms (3.5 ml) of Br_2 dissolved in 5.3 ml of CCl_4. After stirring for an additional 10 minutes the CCl_4 was washed with saturated NaCl, dried over anhydrous Na_2SO_4, and removed *in vacuo* to yield 27.4 gms of a very crude oil which was used without further purification.

IR: ν_{neat} (cm^{-1}): 1730 (s).

4β-Methyl-6β-isopropyl-8aβ-methyl-4aα,5,7,8-tetrahydronaphthalen-3-one (IV-25)

Crude bromoketone (31.66 gms) was dissolved in 450 ml of dimethylformamide (DMF) to which 25 gms of LiCl and 25 gms of Li_2CO_3 were added. An oil bath at 165° was used as a source of heat and the reaction was conducted under an N_2 atmosphere. Despite the precautions, the reaction turned black almost immediately. Removal of the DMF was accomplished by vacuum distillation on a rotary evaporator modified for high (1 mm) vacuum. The residue was taken up in water and $CHCl_3$ and the two phases were separated. The aqueous layer was washed several times with $CHCl_3$. The combined organic layers were washed with water, dried over $MgSO_4$ and the solvent removed *in vacuo*. High-vacuum distillation of the black residue yielded 9.12 gms of eudesmane-type products (Bp: 91-120° at 0.15 mm). VPC analysis (Col. V, temp = 200°) showed a multitude of peaks:

R_t = 4.5 min (15 percent, saturated ketone), 4.8 min (43 percent, desired $\Delta 1$ product), 5.7 min (23 percent, Δ^4-α,β-unsaturated ketone), 6.3 min (10 percent, unidentified).

Column chromatography of the mixture on one kg of activity II neutral alumina yielded 2.34 gms of the desired α,β-unsaturated ketone, which was eluted with 35 percent ether-65 percent pet ether (30°-60°). A fraction (1.35 gms) containing 45 percent of the desired product and 48 percent of the saturated ketone was eluted with 30 percent ether-70 percent pet ether (30°-60°). Overall, isolated yield of α,β-unsaturated ketone from the saturated 3-keto precursor was 21 percent.

IR: $\nu_{neat}^{(cm^{-1})}$: 1665, 1610.

NMR (δ, $CDCl_3$): 0.90 (6H, d, J = 6 Hz, isopropyl methyl), 1.03 (3H, s, angular methyl), 1.12 (3H, d, J = 7 Hz, C-4 methyl), 5.80 and 6.65 (2H, d of d, J = 10 Hz, olefinic H).

Mass Spectrum: M^+_{found} = 220 (47 percent), calculated = 220 ($C_{15}H_{24}O$) base peak (m/e) = 95.

UV: λ_{max}^{MeOH} ($m\mu$): 305 ($\log\varepsilon$ = 1.64), 285 ($\log\varepsilon$ = 1.62)

$\lambda_{max}^{Dioxane}$ ($m\mu$): 335 (shoulder, $\log\varepsilon$ = 1.68).

ORD(MeOH), 25°, c = 3.15 mg/ml) n $\rightarrow \pi^*$: $[\phi]_{700}$ = 2.82, $[\phi]_{589}$ = 2.13 ($[\alpha]_D^{25}$ = 0.96), $[\phi]_{500}$ = 5.64, $[\phi]_{450}$ = 11.28, $[\phi]_{400}$ = 19.74, $[\phi]_{370}$ = 45.13 (shoulder), $[\phi]_{359}$ = 53.59, $[\phi]_{350}$ = 26.79 (inflection), $[\phi]_{340}$ = 0.00, $[\phi]_{329}$ = -43.72 (inflection), $[\phi]_{319}$ = -64.87, $[\phi]_{312}$ = -63.46 (inflection), $[\phi]_{280}$ = -9.87, $[\phi]_{275}$ = -7.05, $[\phi]_{270}$ = -11.28, $[\phi]_{260}$ = -35.26.

(Dioxane, 25°, c = 2.90 mg/ml) n → π*: $[\phi]_{650}$ = 1.52, $[\phi]_{589}$ = 3.03 ($[\alpha]_D^{25}$ = 1.38), $[\phi]_{500}$ = 6.07, $[\phi]_{450}$ = 12.14, $[\phi]_{400}$ = 19.72, $[\phi]_{378}$ = 47.03, $[\phi]_{367}$ = 22.76, $[\phi]_{361}$ = 28.83, $[\phi]_{357}$ = 18.21 (inflection), $[\phi]_{354}$ = 0.00, $[\phi]_{350}$ = -19.72, $[\phi]_{343}$ = -12.14, $[\phi]_{335}$ = -44.00, $[\phi]_{328}$ = -27.31, $[\phi]_{323}$ = -33.38, $[\phi]_{318}$ = -24.27 (shoulder), $[\phi]_{308}$ = -21.24 (inflection), $[\phi]_{295}$ = 0.00, $[\phi]_{275}$ = 21.24, $[\phi]_{257}$ = 0.00, $[\phi]_{250}$ = -24.28.

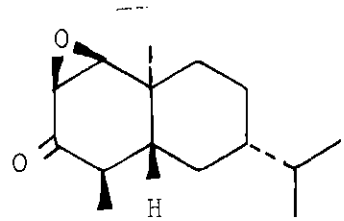
CD (MeOH, 25°, c = 3.15 mg/ml) n → π*: $[\theta]_{382}$ = 0.00, $[\theta]_{350}$ = 28.80 (shoulder), $[\theta]_{340}$ = 34.30, $[\theta]_{328}$ = 23.04 (inflection), $[\theta]_{314}$ = 3.67 (inflection), $[\theta]_{310}$ = 0.00, $[\theta]_{290}$ = -12.57, $[\theta]_{266}$ = -6.81, $[\theta]_{256}$ = -11.26.

(Dioxane, 25°, 2.90 mg/ml) n → π*: $[\theta]_{387}$ = 0.00, $[\theta]_{370}$ = 11.94, $[\theta]_{366}$ = 14.22, $[\theta]_{355}$ = 25.59, $[\theta]_{349}$ = 17.63 (inflection), $[\theta]_{346}$ = 14.79, $[\theta]_{341}$ = 17.63, $[\theta]_{328}$ = 5.12 (shoulder), $[\theta]_{322}$ = 0.00, $[\theta]_{316}$ = -2.84 (shoulder), $[\theta]_{292}$ = -9.10, $[\theta]_{260}$ = -1.71, $[\theta]_{250}$ = -8.53.

Analysis ($C_{15}H_{24}O$): calculated: C 81.76, H 10.98

found: C 81.94, H 10.92.

<u>Δ1-Epoxy-4α-methyl-6β-isopropyl-8aβ-methyl-4aα,5,7,8,-tetrahydro-naphthalen-3-one</u>



To 2.34 gms of α,β-unsaturated ketone (IV-25) in 110 ml of dioxane were added 11 ml of 30 percent H_2O_2 and 11 ml of 5 percent NaOH. The reaction was stirred for 10 minutes at room temperature, then another 11 ml of 30 percent

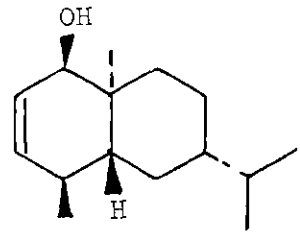
H_2O_2 and 11 ml of 5 percent NaOH were added. Stirring was continued for one additional hour, after which the reaction was poured into one liter of 5 percent NaCl. The aqueous layer was extracted with ether, and the ether was dried over $MgSO_4$ and removed *in vacuo* to yield 2.73 gms of a greenish-yellow oil. VPC analysis (Col. V, temp = 200°) revealed 6 percent of saturated 3-keto precursor, 9 percent of starting α,β-unsaturated ketone, and 81 percent of the desired epoxide (R_t = 5.5 min). Column chromatography on activity II neutral alumina, silica gel, or 10 percent $AgNO_3$ - silica gel would not purify the mixture further.

IR: $\nu_{neat}(cm^{-1})$: 1725.

NMR (δ, $CDCl_3$): 0.85, 0.88, and 0.95 (12H, overlapping singlets and doublets, methyl protons), 3.23 and 3.35 (2H, d of d, J = 4 Hz, C-1 and C-2 protons).

Mass Spectrum: M^+_{found} = 236.1776 (11 percent), calculated = 236.1757 ($C_{15}H_{24}O_2$); base peak (m/e) = 95.

1-Hydroxy-4α-methyl-6β-isopropyl-8aβ-methyl-4aα,5,7,8-tetrahydronaphthalene



The above epoxyketone (2.15 gms) was dissolved in 50 ml of absolute EtOH. To this were added one ml of glacial acetic acid and 5 ml of 85 percent hydrazine hydrate. The reaction was stirred at room temperature for one hour, then 2 ml more of hydrazine hydrate was added. No additional gas evolution was observed and the reaction was quenched by pouring it

into 400 ml of 5 percent NaCl. The mixture was extracted with ether, the ether dried over anhydrous Na_2SO_4 and removed *in vacuo* to yield 2.15 gms of a brownish yellow oil. VPC analysis on two columns (Col. I, temp = 160° , R_t = 1.7 min; Col. V, temp = 200°, R_t = 1.8 min) showed the purity to be greater than 90 percent.

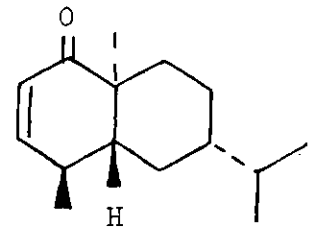
IR: $\nu_{neat}(cm^{-1})$: 3350 (b), 1450, 1020, 1005.

NMR (δ, $CDCl_3$): 0.77, 0.85, 0.93, and 1.07 (12H, overlapping singlets and doublets, methyl protons), 3.33 to 3.67 (1H, complex, C-1 proton), 3.72 (1H, bs, $w_{\frac{1}{2}}$ = 12 Hz, hydroxy proton), 5.47 to 6.00 (2H, complex, olefinic protons).

Mass Spectrum: M^+_{found} = 222.1984, calculated = 222.1998 ($C_{15}H_{26}O$) base peak (m/e) = 85.

4α-Methyl-6β-isopropyl-8aβ-methyl-4aα,5,7,8-tetrahydronaphthalen-1-one (IV-28)

One gram of activated MnO_2, prepared by the Attenburrow procedure,[101] was added to a stirred solution of 250 mg of the above allylic alcohol in 20 ml of cyclohexane. The reaction was stirred at room temperature under an N_2 atmosphere for 23.5 hours after which time the MnO_2 was removed by filtration through Celite 545. Washing the Celite with 600 ml of ether was followed by evaporation of the solvent *in vacuo*. An IR spectrum indicated little or no reaction had taken place.



Repeating the above reaction with Jones reagent[46] led to the

desired product. To a stirred solution of 2.15 gms of the allylic alcohol in 25 ml of acetone, Jones reagent was added dropwise until an excess of reagent was indicated. The chromium salts were then removed by repeated filtration and the residue left after removal of the solvent was taken up in ether. The ether layer was washed with water, dried over $MgSO_4$ and the ether removed in vacuo to yield 1.54 gms of a brownish oil. VPC analysis indicated greater than 95 percent purity (Col. I, temp = 160°, R_t = 2.2 min; Col. II, temp = 160°, R_t = 1.8 min; Col. III, temp = 140°, R_t = 3.7 min; Col. IV, temp = 140°, R_t = 2.0 min; Col. V, temp = 160°, R_t = 2.8 min).

The reaction mixture was then placed on a silica gel column which was prepared using pet ether (30°-60°) as an elutant. Gradient elution, using benzene as the co-solvent, followed and the desired ketone was found to be eluted in the fractions which were approximately 20 percent benzene - 80 percent pet ether. The ketone showed no detectable impurities by VPC. Overall yield of the 1-keto, Δ2-α,β-unsaturated ketone from the 3-keto, Δ1-α,β-unsaturated ketone was 66 percent.

IR: $\nu_{neat}(cm^{-1})$: 1670 (s), 1610 (m).

NMR (δ, $CDCl_3$): 0.88 (6H, d, J = 5 Hz, isopropyl methyl), 1.00 (3H, s, angular methyl), 1.10 (3H, d, J = 8 Hz, C-4 methyl), 5.85 and 6.60 (2H, ABX system, J_{AB} = 10 Hz, J_{AX} = 2 Hz, olefinic protons).

Mass Spectrum: M^+_{found} = 220 (78 percent), calculated = 220 ($C_{15}H_{24}O$) base peak (m/e) = 82.

UV: λ_{max}^{MeOH} (mμ): 225 ($\log\varepsilon$ = 3.90)

$\lambda_{max}^{Dioxane}$ (mμ): 370 (shoulder, $\log\varepsilon$ = 1.30),350 (shoulder, $\log\varepsilon$ = 1.61), 334-326 (broad peak, $\log\varepsilon$ = 1.72).

ORD (MeOH, 25°, c = 2.74 mg/ml) n → π*: $[\varphi]_{700}$ = 9.64, $[\varphi]_{589}$ = 8.03 ($[\alpha]_D^{25}$ = 3.65), $[\varphi]_{500}$ = 11.24, $[\varphi]_{400}$ = 19.27, $[\varphi]_{380}$ = 14.45, $[\varphi]_{369}$ = 25.69, $[\varphi]_{365}$ = 22.48, $[\varphi]_{344-338}$ = 62.63 (broad shoulder), $[\varphi]_{344}$ = 72.26 (shoulder), $[\varphi]_{339}$ = 88.32, $[\varphi]_{334-328}$= 73.87 (broad shoulder), $[\varphi]_{320-308}$ = 51.37 (broad shoulder).

π → π* (c = 0.054 mg/ml): $[\varphi]_{300}$ = 60.23, $[\varphi]_{275}$ = 40.15, $[\varphi]_{260}$ = 200.73, $[\varphi]_{250}$ = 421.53, $[\varphi]_{240}$ = 562.04, $[\varphi]_{235}$ = 381.39, $[\varphi]_{230.5}$ = 0.00, $[\varphi]_{225}$ = -722.63, $[\varphi]_{215}$ = -1445.26, $[\varphi]_{210}$ = -1565.69, $[\varphi]_{205}$ = -883.21, a = 21.28.

(Dioxane, 25°, c = 2.64 mg/ml) n → π*: $[\varphi]_{700}$ = 3.33, $[\varphi]_{589}$ = 6.67 ($[\alpha]_D^{25}$ = 3.03), $[\varphi]_{500}$ = 10.00, $[\varphi]_{400}$ = 13.33, $[\varphi]_{380}$ = 5.00, $[\varphi]_{366}$ = 53.33, $[\varphi]_{359}$ = 46.67, $[\varphi]_{350}$ = 85.00, $[\varphi]_{342}$ = 45.00, $[\varphi]_{336}$ = 61.67, $[\varphi]_{326}$ = 18.33, $[\varphi]_{322}$ = 21.67, $[\varphi]_{315}$ = 5.00, $[\varphi]_{300}$ = 20.00, $[\varphi]_{290}$ = 48.33.

CD (MeOH, 25°, c = 2.74 mg/ml): n → π*: $[\theta]_{385}$ = 0.00, $[\theta]_{374}$ = -5.06 (shoulder), $[\theta]_{366}$ = -6.50 (shoulder), $[\theta]_{356}$ = -14.21, $[\theta]_{346}$ = -6.26, $[\theta]_{345}$ = -6.50, $[\theta]_{347}$ = 6.26, $[\theta]_{343}$ = -6.50, $[\theta]_{341}$ = -5.30 (shoulder), $[\theta]_{338}$ = 0.00, $[\theta]_{330}$ = 7.71 (shoulder), $[\theta]_{320}$ = 13.48, $[\theta]_{309}$ = 7.71, $[\theta]_{294}$ = 0.00.

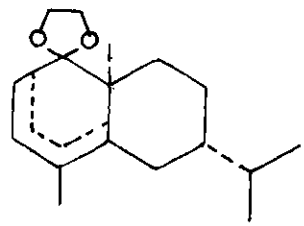
π → π* (c = 0.054 mg/ml): $[\theta]_{254}$ = 0.00, $[\theta]_{228}$ = 589.93, $[\theta]_{215}$ = 0.00, Γ = 19 mμ.

(Dioxane, 25°, c = 2.64 mg/ml)n → π*: $[\theta]_{388}$ = 0.00, $[\theta]_{371}$ = -13.25, $[\theta]_{362}$ = -0.62, $[\theta]_{355}$ = -9.25, $[\theta]_{352}$ = 0.00, $[\theta]_{346}$ = 15.74, $[\theta]_{342}$ = 12.75 (shoulder), $[\theta]_{338}$ = 11.00, $[\theta]_{330}$ = 19.99, $[\theta]_{328}$ = 19.74 (shoulder), $[\theta]_{324}$ = 12.74, $[\theta]_{321}$ = 13.49, $[\theta]_{308}$ = 1.75 (shoulder), $[\theta]_{307}$ = 0.00.

Analysis ($C_{15}H_{24}O$): calculated: C 81.76, H 10.98

found: C 81.66, H 10.72.

Ketalization of α,β-unsaturated Ketone (IV-28)



To 25 ml of benzene was added one ml of ethylene glycol, 50 mg of PTSA and 231 mg of the α,β-unsaturated ketone (IV-28). The mixture was refluxed under a Dean Stark trap for 16.5 hours after which time the benzene was washed with 5 percent $NaHCO_3$, dried over Na_2SO_4 and removed in vacuo to yield 267 mg (96 percent) of a 1:3.4:3.7 mixture of the three possible ketals by VPC (Col. I, temp = 160°, R_t = 3.8, 4.3 and 4.7 min, respectively). NMR indicated that the predominant product was the Δ3 ketal, followed by the Δ4 ketal and then the Δ2 ketal.

IR: ν_{neat} (cm^{-1}): 1650 (m), 940 (m), 875 (m).

NMR (δ, $CDCl_3$): 1.57 (~2H, bs, $w_{\frac{1}{2}}$ = 5 Hz), 3.88 (4H, bs, $w_{\frac{1}{2}}$ = 4Hz), 5.25 (~0.3H, bs, $w_{\frac{1}{2}}$ = 8Hz).

Mass Spectrum: M^+_{found} = 264 (68 percent), calculated = 264 ($C_{17}H_{28}O_2$); base peak (m/e) = 99.

Analysis ($C_{17}H_{28}O_2$): calculated: C 77.22, H 10.67

found: 77.10, H 10.58.

Irradiation of Δ2, Δ3, Δ4 Ketal mixture[102]

A solution of 260 mg of ketal mixture was prepared in 65 ml of benzyl alcohol and outgassed vigorously with N_2 for one hour. Irradiation then followed using a 450 watt Hanovia Hg lamp with a Pyrex filter. The heat of the lamp was removed using cold water coolant. After six hours, the benzyl alcohol was removed in vacuo to give a brown polymeric-looking material. When MeOH at zero degrees was used as a coolant, the results were similar, but the polymeric material was white in color. A portion of the polymer was soluble in organic solvents. VPC analysis of the soluble material showed it to be mostly two compounds (Col. I, temp = 160°, R_t = 4.2 min and 4.5 min).

Chromatography of the mixture on a 10 percent $AgNO_3$ - silica gel column using a pet ether - benzene gradient elution followed. Two relatively pure compounds were isolated, both with the same VPC retention time (3 min under the above conditions) but with different column retention times.

The compound with the longer column retention time was shown to be the pure Δ3 ketal (46.2 mg).

NMR (δ, $CDCl_3$): 0.82 (3H, s, angular methyl), 0.85 (6H, d, J = 6 Hz, isopropyl methyl), 1.57 (3H, bs, $w_{\frac{1}{2}}$ = 3.5 Hz, C-4 methyl), 3.90 (4H, bs, $w_{\frac{1}{2}}$ = 3 Hz, ketal protons), 5.23 (1H, bs, $w_{\frac{1}{2}}$ = 9 Hz, olefinic proton).

The compound with the shorter column retention time was the Δ4 ketal (89.8 mg).

NMR (δ, $CDCl_3$): 1.57 (3H, bs, $w_{\frac{1}{2}}$ = 6 Hz, C-4 methyl), 3.90 (4H, s, ketal protons).

No exocyclic double bond material was noted.

In a second photolysis, run as before, using the same amounts, the isolated mixture of $\Delta 2$ and $\Delta 3$ ketals (102.3 mg) was hydrolyzed with a mixture of 12 ml of wet acetone containing one ml of 10 percent aqueous HCl. The recovered mixture of ketones showed only one peak on the VPC (Col. I, temp = 160°, R_t = 2.0 min); however, column chromatography on 10 percent $AgNO_3$ - silica gel as before, yielded two components. The first compound was the $\Delta 4$, 1-keto compound.

IR: $\nu_{neat}(cm^{-1})$: 1715 (s).

NMR (δ, $CDCl_3$): 1.48 (3H, bs, C-4 methyl).

The second compound was the $\Delta 3$, 1-keto compound (28.2 mg).

IR: $\nu_{neat}(cm^{-1})$: 3040 (w), 3070 (w), 1715 (s).

NMR (δ, $CDCl_3$): 1.63 (3H, bs, C-4 methyl), 4.90 (1H, bs, olefinic proton). Again, no exocyclic double bond compound was noted in the reaction mixture.

A third photolysis was run using 154.5 mg of α,β-unsaturated ketone (IV-28) dissolved in 100 ml of benzyl alcohol. After outgassing for one hour with N_2, a 450 watt, quartz filtered Hanovia lamp was turned on. Again water was used as a coolant. After six hours the benzyl alcohol was removed <u>in vacuo</u> and the residue was chromatographed on 10 percent $AgNO_3$ - silica gel as before. The results proved no different than for the Pyrex filtered case, and a lot more

polymeric material was observed. No exocyclic double bond was observed in the IR or NMR.

Reaction of α,β-unsaturated Ketone (IV-28) with NBS

To 5 ml of CCl_4 was added 160 mg of α,β-unsaturated ketone (IV-28) and 130 mg of freshly recrystallized N-bromosuccinimide (NBS).[103] The reaction was irradiated with a 250 watt Hg (Hanovia) lamp for two hours. The ketone was stable to irradiation with this lamp for at least one hour when no NBS was added. The CCl_4 was removed in vacuo and a VPC showed one major product (Col. I, temp = 160°, R_t = 3.2 min), some starting ketone and some high retention time material. An NMR of the crude reaction mixture indicated loss of double bond protons and possible formation of a methyl group on a double bond (δ 1.18). The material was then chromatographed on a 9 gm silica gel column, prepared using pet ether (30°-60°) as a solvent. Elution of the column with increasing percentages of benzene led to the isolation of the major product in small amounts. An IR of this material indicated no carbonyl. No attempt was made to identify this product further.

The reaction was repeated without the use of a light source other than room fluorescent lighting.[104] To 46.3 mg of α,β-unsaturated ketone (IV-28) was added 5 ml of CCl_4 and 45 mg of N-bromosuccinimide. The CCl_4 was refluxed under an N_2 atmosphere and VPC samples were taken periodically. After five minutes (usual NBS reaction time) no reaction had taken place. After 15 minutes some new material was appearing (Col. I, temp = 160°, R_t = 2.3 min

(10 percent), R_t = 7.2 min (2 percent)). Both peaks gradually increased as the refluxing continued. After two hours the composition was (by VPC, as above) 47 percent starting ketone, 38 percent new low R_t product and 14 percent new high R_t product. The solution was cooled to room temperature and the precipitated succinimide was removed by filtration. NMR ($CDCl_3$) revealed, as expected, peaks due to starting material, and new peaks at δ = 1.93, 1.95, 4.22, 4.30 and many smaller peaks not due to starting material. An IR (CCl_4) showed peaks at 1750 cm^{-1}, 1725 cm^{-1} and 1680 cm^{-1}. The reaction was restarted by dissolving the recovered material in 5 ml of CCl_4 and adding 50 mg more NBS. At 6.5 hours the VPC showed 11 percent starting ketone, 46 percent low R_t product and 40 percent high R_t product. The product composition by VPC remained unchanged during the next 3.5 hours and the reaction was stopped after 10 hours total reaction time had elapsed. The reaction was worked up as before.

A preparative thin layer chromatograph on a Brinkmann pre-coated TLC plate (Sil-G-100 UV_{254} coated 1.0 mm, 20 x 20 cm) using benzene as the eluting solvent revealed eight bands (R_f = 0.00 (10.5 mg), 0.07, 0.25 (8.9 mg), 0.37 (8.9 mg), 0.45 (8.4 mg), 0.51 (5.3 mg), 0.58 (5.6 mg), 0.72 (6.0 mg), and 0.84 (3.8 mg)). A VPC (column conditions as above) of the R_f = 0.00 material showed it to contain either non-volatile material or highly volatile material since no significant peaks were observed. A mass spectrum showed an M^+ = 280, no bromine present and a base peak at m/e = 159. IR

revealed a band characteristic of a saturated carbonyl (1700 cm^{-1})[105] but this band was not well resolved (all IR spectra taken in this study were done in CCl_4 solution). VPC analysis of the R_f = 0.07 material showed it to have material with a retention time of 2.3 minutes. Mass spectral analysis showed no bromine, an M^+ = 218 and a base peak at m/e = 175. IR revealed a conjugated ketone at 1660 cm^{-1} and a double bond at 1620 cm^{-1}.[105] The R_f = 0.25 material had a similar VPC, but other peaks were also observed. The IR and mass spectrum were similar to the previous one (M^+ = 218, base peak however was m/e = 43). The R_f = 0.37 material had some of the R_t = 2.3 min material, but was mostly R_t = 7.2 min material. Mass spectral analysis revealed that it was a mono-bromide (M^+ = 297, base peak was m/e = 218, probably due to the contaminant).[106] IR showed absorptions due to a non-conjugated ketone (1725 cm^{-1}) and a conjugated ketone (1675 cm^{-1}).[105] The R_f = 0.45 material showed no strong peak in the VPC, but several weak ones were observed. The mass spectrum had a multitude of peaks, showing that several compounds were present. Bromine was evident in several of the regions of the mass spectrum. However, the IR was relatively clean, showing a non-conjugated ketone absorption at 1725 cm^{-1} and double bond bands at 3100 cm^{-1} and 1675 cm^{-1}.[105] The R_f = 0.51 material had a broad high-R_t peak in the VPC, but also showed a sharp peak at R_t = 6.8 minutes. Mass spectral analysis showed the latter peak to be a mono-bromide,[106] M^+ = 299, with a base peak at m/e = 95. IR showed a non-conjugated ketone absorption at 1725 cm^{-1} and double bond absorptions (3020 and 1680

cm^{-1}).[105] The R_f = 0.58 material had mostly a large, broad high-R_t peak (15.9 min) in the VPC. Mass spectral analysis showed this to be a mono-bromide with an M^+ = 301 (base peak (m/e) = 72).[106] The infrared spectrum was not well resolved in the carbonyl region but did show a shoulder at 1725 cm^{-1}, a peak at 1690 cm^{-1} and one at 1675 cm^{-1}.[105] The R_f = 0.72 material had no significant peaks in the VPC. Mass spectral analysis showed fragmentations due to more than one compound. Bromine was present in some regions.[106] Peaks at m/e's of 298, 300 and 302 were observed. The IR showed peaks due to a non-conjugated carbonyl (1725 cm^{-1}), conjugated carbonyl (1690 cm^{-1}) and double bonds (1675 cm^{-1}).[105] The last fraction, R_f = 0.84, again showed no significant peaks in the VPC. The mass spectrum revealed only low m/e (less than 120) material and the IR showed no carbonyl.

Attempted Base Deconjugation of α,β-unsaturated Ketone (IV-28)

To 25 ml of dry t-BuOH was added 130 mg of t-BuOK and 40 mg of α,β-unsaturated ketone (IV-28).[107] The reaction mixture was stirred for one hour at room temperature under an N_2 atmosphere after which it was poured into 100 ml of water. Extraction of the aqueous layer with ether, drying of the ether over $MgSO_4$ and removal of solvent *in vacuo* left 31.3 mg of starting material as shown by IR comparison.

The reaction was repeated as above, using the recovered material, but quenching was done by the rapid addition of 15 ml of 10 percent HOAc via a syringe and rapid pouring of the reaction mixture into 50 ml of 5 percent $NaHCO_3$. The usual ether extraction procedure followed. Yield was 25.1 mg of starting material.

Again the reaction was repeated using the above recovered material, 500 mg of t-BuOK in 25 ml of t-BuOH and a reaction time of 68 hours at room temperature. Workup was as in the above case. IR of the recovered material showed two carbonyls, one due to starting material and one in the same location as the previously prepared 1-keto, Δ3 double bond compound.

The reaction was again repeated using 23.5 mg of α,β-unsaturated ketone (IV-28) in 25 ml of dry dioxane and 500 mg of a 57 percent dispersion of NaH in mineral oil, which were stirred for 18 hours at room temperature. Quenching and workup were done as above. A preparative TLC on silica gel using 10 percent benzene - 10 percent ether - 80 percent hexane as a solvent system gave one major spot (R_f = 0.00) and two minor spots (R_f = 0.10 and 0.40). An IR of the R_f = 0.00 material showed no carbonyl. The material looked polymeric to the eye.

Another attempt was made using the conditions of the last t-BuOH, t-BuOK reaction, but the reaction was run at 65° for 66 hours. Workup was as before and an IR of the product showed it to be the same as with the previous room temperature reaction.

A final attempt at improving the yield was made using trityl lithium as a base. To 600 mg of triphenylmethane in 10 ml of dry benzene at room temperature under an N_2 atmosphere, was added 1.6 ml of 1.25 M BuLi in pentane. The reaction was stirred at room temperature for 15 minutes, then 25 mg of the α,β-unsaturated ketone (IV-28) in 10 ml of dry benzene was added. After 16 hours the reaction was poured into 25

ml of 10 percent HOAc, then 100 ml of 5 percent $NaHCO_3$, and extracted with pentane. Repeated washings with pentane gave a product which was free of triphenylmethane. This material showed no carbonyl in the IR and visually looked polymeric.

Since the only reaction condition that yielded any deconjugation was t-BuOK in t-BuOH for 67 hours, this reaction was repeated on a larger scale. To 2.0 gms of t-BuOK in 90 ml of dry t-BuOH was added 111.6 mg of α,β-unsaturated ketone (IV-28). The reaction was stirred for 67 hours at room temperature and worked up in the usual manner. An IR spectrum indicated most of the carbonyl was deconjugated; however, the IR was not well resolved. The material was chromatographed on 12 gms of 10 percent $AgNO_3$ - silica gel using a hexane - benzene linear gradient. From the chromatography, 53.3 mg of material that was 90 percent deconjugated by VPC was collected. An additional 16.9 mg of material that was approximately 50 percent deconjugated was collected. An NMR spectrum of the former fraction showed that the deconjugated material contained polymer (aromatic absorptions and intense absorptions in odd places). This fraction was hot box distilled (Bp: 65° at 0.1 mm) to yield 17.9 mg of material which showed (by NMR) approximately 10 percent α,β-unsaturated ketone, 10 to 20 percent polymer and the rest deconjugated material.

Various amide bases were then tried in an attempt to deconjugate the ketone.[108] The general procedure involved adding 0.2 ml of 1.34 M BuLi in pentane to a stirred solution of 35μl of the amine used to generate the amide, in 5 ml of dimethoxyethane (DME) under an N_2

atmosphere. All amines were distilled from CaH prior to use. The reaction between the amine and the butyllithium was conducted with slow warming from -40° to -25° using a reaction time of 15 minutes. After this time, approximately 40 to 50 mg of ketone (IV-28) dissolved in 5 ml of dry DME was added dropwise. The temperature was maintained at $-20^{\circ} \pm 2^{\circ}$ during this phase of the reaction, which took one hour (15 minutes to add the ketone plus 45 minutes of additional reaction time). Then the reaction was allowed to warm to room temperature for 15 minutes and one ml of water was added in 0.5 seconds. Workup involved removing the amine in vacuo and taking up the residue in ether. The organic layer was separated, dried over $MgSO_4$ and the solvent removed in vacuo. With diisopropylamine (used 47.1 mg of ketone) no reaction was observed by VPC analysis or by NMR. Repetition with diethylamine (used 20.3 mg of ketone) also showed no reaction under similar analysis conditions. Finally, n-propylamine was tried and again no reaction was observed.

Attempted Formation of the Enol Acetate of (IV-28)

To 51.3 mg of α,β-unsaturated ketone (IV-28) was added 10 ml of isopropenyl acetate and a trace of p-toluenesulfonic acid. The solution was refluxed under a Dean Stark trap for 24 hours. The reaction mixture was cooled to room temperature and it was extracted with 5 percent $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed in vacuo. VPC analysis (Col. I, temp = 160°) revealed a multitude of peaks, none in any predominance. IR and NMR analysis showed little, if any, enol acetate.

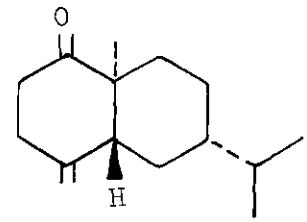
Enolate trapping was the next method tried.[108] To 5 ml of dry dimethoxyethane (DME) was added 180 µl of 1.37 M BuLi in pentane. The solution was stirred for 10 minutes under an N_2 atmosphere at -17°. Then 35 µl of freshly distilled (from CaH) diisopropylamine was added. The amide formation reaction was conducted at -17° for five minutes, then 44.8 mg of α,β-unsaturated ketone (IV-28) in 5 ml of dry DME was added dropwise. The reaction mixture was stirred for 35 minutes from the start of the ketone addition, after which time the solution was allowed to warm to room temperature. Rapid (0.5 seconds) addition of one ml of Ac_2O followed and the reaction was stirred for five minutes. Excess acetic anhydride was removed by the addition of 5 percent $NaHCO_3$ until basic. The organic layer was separated and the aqueous layer was washed with ether. The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. VPC and IR showed no reaction.

Acid conditions were then tried again. To 10 ml of CCl_4 were added 55.8 mg of α,β-unsaturated ketone (IV-28), 5 ml of Ac_2O, and a trace of 70 percent $HClO_4$ (added last).[108] The reaction mixture was stirred at room temperature and followed by VPC (Col. I, temp = 160°). While the ketone disappeared at a reasonable rate, nothing new appeared in any major amount, as shown by VPC. After three hours, the reaction mixture was poured into water and the acetic anhydride was neutralized with solid $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, and the solvent removed by distillation. An NMR of the residue was inconclusive; however, no enol acetate was formed.

In order to further understand what was happening during the reaction, the α,β-unsaturated ketone (IV-28) was dissolved in CCl_4 and placed in an NMR tube. To it was added a trace of 70 percent $HClO_4$. Before the perchloric acid was added, an NMR was taken and all subsequent NMR's were taken at the same machine settings (primarily Rf field and spectrum amplitude). At t = 0 (no $HClO_4$ added) the following peaks were observed in the methyl region (numbers following the δ values are peak height in millimeters from the base line): 0.85 (108), 0.93 (183), 1.02 (137), 1.07 (128), and 1.18 (114). At t = 1.25 hours a slight decrease in the vinyl region was noted (less than 5 percent), a strong peak at δ 7.67 (bs, $w_{\frac{1}{2}}$ = 6 Hz) was noted, and the following methyl peaks were observed: 0.85 (107), 0.93 (192), 1.03 (169), 1.07 (123), and 1.20 (111). At t = 2.25 hours, no peak at δ 7.67 was observed, nor was any low field peak observed at 1000 Hz sweep width, the vinyl protons still were characteristic of an ABX system and had undergone no change in intensity, and the methyl region took the following form: 0.85 (126), 0.93 (198), 1.03 (193), 1.07 (114), and 1.20 (118). At t = 4 hours, no change was noted in the vinyl region, and the peak at δ 1.07, which had been clearly separated from the peak at δ 1.03, had coalesced with the latter. The methyl region now took the following form: 0.85 (137), 0.93 (173), 1.03 (171), and 1.20 (115). The final observation was taken at t = 6 hours and little change was evident from the spectrum at t = 4 hours. The vinyl region still maintained the same ABX coupling pattern and intensities as at t = 0; however, from t = 0 to the t = 6 hours spectra a significant change in the methyl region

had taken place. At t = 6 hours, it took the following form: 0.85 (121), 0.93 (174), 1.03 (169) and 1.20 (115).

4-Methylene-6β-isopropyl-8aβ-methyl-2,3,4aα,5,7,8-hexahydronaphthalen-1-one



From 589.6 mg of the α,β-unsaturated ketone (IV-28), 632.6 mg of the ketal mixture was prepared as before using 4 ml of ethylene glycol, 75 ml of benzene, and 100 mg of p-toluenesulfonic acid as a catalyst. The ratio of products was the same as before. Chromatography of the mixture on a 10 percent $AgNO_3$ - silica gel column yielded 269.1 mg of the pure Δ3 double bond ketal.

IR: $\nu_{neat}(cm^{-1})$: 1630 (m).

NMR (δ, $CDCl_3$): 0.82 (3H, s, angular methyl), 0.83 (6H, d, J = 7 Hz, isopropyl methyl), 1.58 (3H, s, C-4 methyl), 3.83 (4H, s, ketal protons), 5.22 (1H, bs, $w_{\frac{1}{2}}$ = 9 Hz, olefinic proton).

Mass Spectrum: M^+ = 264 (33 percent), calculated = 264 ($C_{17}H_{28}O_2$) base peak (m/e) = 32.

The deconjugated ketal was then dissolved in 15 ml of dichloroethane to which was added 250 mg of 85 percent m-chloroperbenzoic acid. After 18.3 hours of stirring at room temperature, the dichloroethane was diluted with 50 ml of $CHCl_3$ and the organic layer was extracted with 5 percent $NaHCO_3$. The aqueous layer was washed with chloroform, and the combined organic layers were dried over Mg SO_4 and the solvent removed *in vacuo*. VPC analysis (Col. I, temp = 160°) revealed 4

percent starting material remaining (R_t = 4.7 min) and new peaks for the β-epoxide (R_t=5.5 min, 24 percent) and the α-epoxide (R_t = 7.0 min, 68 percent). This material was not further purified.

IR: $\nu_{neat}(cm^{-1})$: 1380.

NMR (δ,$CDCl_3$): 0.90 (6H, d, J = 6 Hz, isopropyl methyl), 0.95 (3H, s, angular methyl), 1.25 (3H, s, C-4 methyl), 3.00 (~1H, t, J = 3 Hz, C-3 proton), 3.95 (4H, complex, ketal protons).

Opening of the epoxide was accomplished by dissolving the above mixture of epoxides in 25 ml of dry tetrahydrofuran under an N_2 atmosphere and adding 75 mg of lithium aluminum hydride. The reaction mixture was refluxed for 16.75 hours, allowed to cool slowly for one hour, then cooled on an ice bath for one-half hour, after which time 0.1 ml of water was added, immediately followed by 0.1 ml of 10 percent NaOH and 0.3 ml of water. Removal of the lithium salts by filtration followed. The organic layer was washed with water, then with saturated salt solution, dried over $MgSO_4$, and the solvent removed in vacuo. VPC analysis (Col. I, temp = 160°) showed the mixture to be complex, with the alcohol retention times very close to those of the epoxide. No α-epoxide was left; however, a peak with R_t = 7.0 min appeared. The NMR showed little epoxide remaining.

IR: $\nu_{neat}(cm^{-1})$: 3400 (b).

NMR (δ, C_6D_6): 0.90 (6H, d, J = 5 Hz, isopropyl methyl), 0.95 (3H, s, angular methyl), 1.10 (3H, s, C-4 methyl), 2.67 (1H, bs, $w_{\frac{1}{2}}$ = 10 Hz, alcoholic proton), 3.57 (4H, complex, ketal protons).

The alcohol mixture was then dissolved in 15 ml of dry pyridine

to which 2 ml of phosphorousoxychloride was added.[95] Stirring at room temperature continued for five hours, after which time the reaction mixture was cooled for 30 minutes on an ice bath. Ice cold water (10 ml) was slowly added dropwise (over approximately 20 min), and then the reaction mixture was poured into 100 ml of ice water and extracted repeatedly with ether. The ether layer was washed with 10 percent HCl till acid, 5 percent $NaHCO_3$ till basic, dried over $MgSO_4$ and the ether removed *in vacuo*. The recovered reaction product (183.9 mg) contained exocyclic ketal with approximately 30 percent other products (mostly unreacted alcohols). Little (less than 10 percent) ketal hydrolysis had occurred. Chromatography on 30 gms of 10 percent $AgNO_3$ - silica gel using a linear hexane - benzene gradient yielded 39.3 mg of pure exocyclic ketal. This was hydrolyzed by stirring in 15 ml of acetone, to which 1.5 ml of water and one ml of 10 percent HCl had been added, for ten hours. Addition of 5 ml of 5 percent $NaHCO_3$ quenched the reaction and the acetone was removed *in vacuo*. The residue was taken up in ether and water and the organic layer was separated. Ether washings of the aqueous layer were then added to it and the combined organic layers were dried over $MgSO_4$ and the ether removed *in vacuo* to yield 25.0 mg of exocyclic ketone. VPC analysis revealed that the material was 80 percent pure: Col. I, temp = 160°, R_t = 2.0 min (90 percent), 3.5 min (3 percent ketal), and 4.0 min (7 percent ketal); Col. II, temp = 140°, R_t = 2.8 min (10 percent ketone impurity), 3.2 min (85 percent exocyclic ketone),and 3.8 min (5 percent ketal impurity); Col. IV, temp = 140°, R_t = 2.0 min (10 percent ketone impurity), 2.3 min (80 percent exocyclic ketone), 2.8 min (3 percent ketal impurity),

and 4.3 min (7 percent ketal impurity); Col. V, temp = 160°, R_t = 5.2 min (10 percent), 6.3 min (80 percent exocyclic ketone), and 7.0 min (10 percent). The material was then chromatographed on 5 gms of 10 percent $AgNO_3$ - silica gel. The pure exocyclic ketone was eluted in the 20 percent benzene - 80 percent hexane fraction. A purity of greater than 95 percent was determined on Col. IV, which the above VPC study showed to be the best column for the separation of this compound mixture. The yield was 11.2 mg (2 percent) from starting α,β-unsaturated ketone (IV-28).

Bp: 50° at 0.05 mm (hot box).

IR: $\nu_{neat}(cm^{-1})$: 3085, 1720, 1660, 1470, 1380, 1375, 1280, 1250, and 890.

IR: $\nu_{neat}(cm^{-1}$, reported for canarone): [24] 3080, 1700, 1640, 1420, 1375, 1360, 1260, 1225, 1183, 1160, and 890.

NMR (δ, $CDCl_3$): 0.87 (6H, d, J = 6 Hz, isopropyl methyl), 0.88 (3H, s, angular methyl), 4.68 (1H, bs, $w_{\frac{1}{2}}$ = 5Hz, olefinic proton), 4.92 (1H, bs, $w_{\frac{1}{2}}$ = 5 Hz, olefinic proton).

Mass Spectrum: M^+_{found} = 220.1845 (38 percent), calculated = 220.1827 ($C_{15}H_{24}O$); base peak (m/e)= 43.

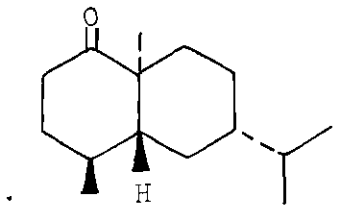
UV: λ^{MeOH}_{max} (mμ): 275 (log ε = 2.45), 254 (log ε = 2.38, inflection), 248 (log ε = 2.34, inflection).

ORD (MeOH, 25°, c = 2.8 mg/ml): $[\phi]_{700}$ = -39, $[\phi]_{600}$ = -43, $[\phi]_{589}$ = -43 ($[\alpha]^{25}_D$ = -20), $[\phi]_{500}$ = -71, $[\phi]_{450}$ = -86, $[\phi]_{400}$ = -110, $[\phi]_{350}$ = -157, $[\phi]_{314}$ = -361 (shoulder), $[\phi]_{300}$ = -432 (inflection), $[\phi]_{284}$ = -519 (peak), $[\phi]_{250}$ = -39 (trough).

CD (MeOH, 25°, c = 2.8 mg/ml): $[\theta]_{400} = 0$, $[\theta]_{380} = 62$, $[\theta]_{307} =$ 53 (inflection), $[\theta]_{306} = 0$, $[\theta]_{300} = -74$ (inflection), $[\theta]_{265} =$ -571 (peak), $[\theta]_{230} = -147$.

4α-Methyl-6β-isopropyl-8aβ-methyl-2,3,4aα,5,7,8-hexahydronaphthalen-1-one

To 10 ml of absolute EtOH was added 100 mg of the α,β-unsaturated ketone (IV-28) and 50 mg of 5 percent Pd/C. The compound was allowed to react with H_2 at atmospheric pressure until no further uptake of hydrogen was indicated, at which point the catalyst was removed by filtration through Celite 545 and the solvent was removed in vacuo to yield 89 mg (88 percent) of the saturated ketone. Distillation in a hot box (55° at 0.05 mm) yielded 76 mg of saturated ketone. VPC analysis showed a single peak on Col. I at 130° (R_t = 2.0 min) and a single peak on Col. IV at 130° (R_t = 2.7 min). However, on Col. III at 140° a small shoulder could be detected (R_t = 2.5 min). The shoulder amounted to less than 10 percent and was probably due to the C-4 epimer (see below). This shoulder was also detectable on Col. II at 120° (R_t = 6.5 min).



IR: ν_{neat} (cm^{-1}): 1720.

NMR (δ, $CDCl_3$): 0.88 (6H, d, J = 5 Hz, isopropyl methyl), 0.93 (3H, s, angular methyl), 1.02 (3H, d, J = 7 Hz, C-4 methyl).

Mass Spectrum: M^+_{found} = 222 (66 percent), calculated = 222 ($C_{15}H_{26}O$) base peak (m/e) = 43.

UV: λ_{max}^{MeOH} (mμ): 274-281 (logε = 2.26, broad peak).

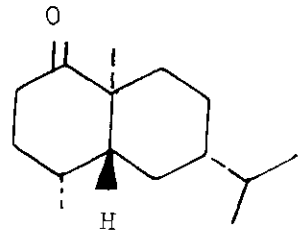
ORD (MeOH, 25°, c = 0.304 mg/ml): $[\phi]_{589}$ = -21.94 ($[\alpha]_D^{25}$ = -9.87), $[\phi]_{450}$ = -56.52, $[\phi]_{400}$ = -87.78, $[\phi]_{350}$ = -190.19, $[\phi]_{325}$ = -372.06, $[\phi]_{306}$ = -651.03, $[\phi]_{300}$ = -504.73, $[\phi]_{287}$ = 0.00, $[\phi]_{275}$ = 387.69, $[\phi]_{273}$ = 468.16, $[\phi]_{250}$ = 468.16, $[\phi]_{200}$ = 0.00, a = -11.19.

CD (MeOH, 25°, c = 0.304 mg/ml): $[\theta]_{327}$ = 0.00, $[\theta]_{291}$ = -868.10, $[\theta]_{235}$ = 0.00, Γ = 33 mμ.

Analysis ($C_{15}H_{26}O$): calculated: C 81.02, H 11.78

found: C 80.92, H 11.89.

4β-Methyl-6β-isopropyl-8aβ-methyl-2,3,4aα,5,7,8-hexahydronaphthalen-1-one



The Δ3 ketal prepared previously and isolated from a photolysis reaction (91 mg) was hydrogenated as above using 70 mg of 5 percent Pd/C as a catalyst. Removal of the ketal protecting group was accomplished by stirring the hydrogenation product in 12 ml of wet acetone to which one ml of 10 percent HCl was added. The reaction was followed by VPC and the ketal had disappeared after 20 minutes. Solid $NaHCO_3$ was added to destroy the excess acid and the solvent was removed *in vacuo*. The residue was taken up in water - ether. The ether layer was separated, dried over $MgSO_4$ and the ether removed *in vacuo* to yield 59.8 mg of crude ketone which was chromatographed on 5 gms of silica gel. The desired ketone (38.6 mg, 51 percent) was

eluted with 20 percent benzene - 80 percent hexane. The compound showed a single peak on all VPC columns used: Col. I, temp = 160°, R_t = 2.3 min; Col. II, temp = 120°, R_t = 7.2 min; Col. III, temp 140°, R_t = 1.8 min; Col. IV, temp = 130°, R_t = 2.7 min. A mixed injection of this compound with its C-4 methyl epimer on Col. II gave two peaks, the second peak occurring at the same place as the shoulder on the C-4 α-methyl compound (see above).

Bp: 55° at 0.05 mm (hot box).

IR: $\nu_{neat}(cm^{-1})$: 1740 (m, shoulder), 1700 (s).

NMR (δ, $CDCl_3$): 0.88 (6H, d, J = 6 Hz, isopropyl methyl), 1.00 (3H, s, angular methyl), 1.23 (3H, d, J = 7 Hz, C-4 methyl).

Mass Spectrum: M^+_{found} = 222.2002 (70 percent), calculated = 222.1984 ($C_{15}H_{26}O$); base peak (m/e) = 43.

UV: $\lambda^{MeOH}_{max}(m\mu)$: 267 ($\log\varepsilon$ = 2.31).

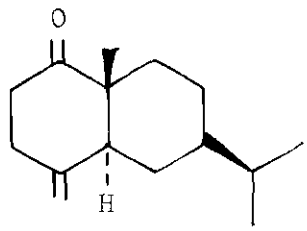
ORD (MeOH, 25°, c = 0.161 mg/ml): $[\phi]_{589}$ = -55.08($[\alpha]^{25}_D$ = -24.77), $[\phi[_{400}$ = -13.77, $[\phi]_{375}$ = -27.54, $[\phi]_{350}$ = -61.96, $[\phi]_{325}$ = -192.77, $[\phi]_{303}$ = -468.16, $[\phi]_{300}$ = -454.39, $[\phi]_{288}$ = 0.00, $[\phi]_{275}$ = 688.46, $[\phi]_{263}$ = 743.54, $[\phi]_{250}$ = 674.69, $[\phi]_{240}$ = 605.85, a = -12.12.

CD (MeOH, 25°, c = 0.161 mg/ml): $[\theta]_{321}$ = 0.00, $[\theta]_{287}$ = -898.74, $[\theta]_{240}$ = 0.00, Γ = 29 mμ.

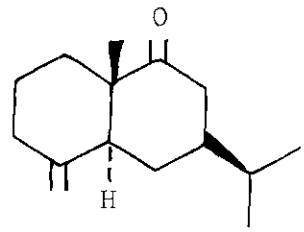
Analysis ($C_{15}H_{26}O$): calculated: C 81.02, H 11.78
found: C 80.76, H 11.74.

Results and Discussion

As mentioned in the introduction section, Bhattacharyya has proposed two structures for canarone (IV-1)[24] and (IV-2)[94]. Synthesis of



IV-1



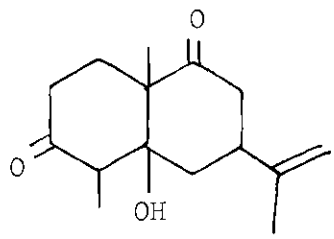
IV-2

(IV-2) has been reported by Zalkow and Lacoume and has been shown to differ spectrally from natural canarone.[95] We wish to report the synthesis of (IV-1) as its enantiomer and comment on its spectral differences from natural canarone.

Any synthetic pathway must be discussed in terms of its overall approach. Ideally, a synthesis should be short, efficient, and if possible introduce new synthetic methods, or extend the usefulness of previous model systems. In our opinion, the synthetic approach taken to (IV-1) fulfills these desires.

One requirement of the synthetic route was that it be readily generalizable toward other synthetic intermediates which would be useful in the synthesis of other sesquiterpenes. A general route has been suggested by Zalkow and Lacoume in their previous synthesis of (IV-2).[95] Reaction of hydroxycarvone with ethyl vinyl ketone (EVK) in the presence of pyridine and then pyrrolidine gave the desired

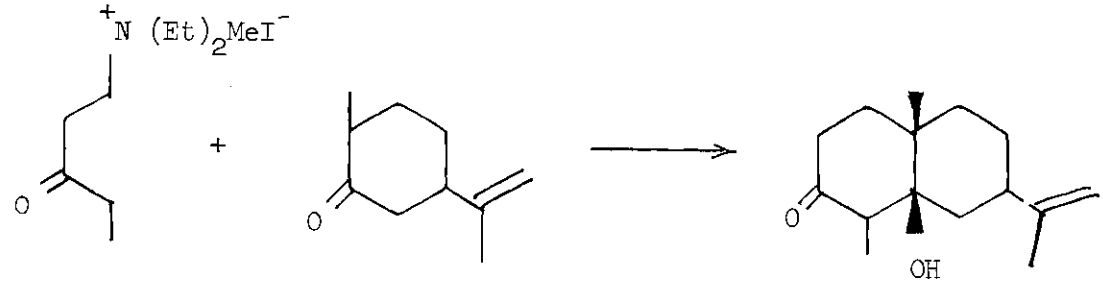
Robinson annelation product (IV-6) which was transformed into (IV-2) as discussed previously. In an analogous manner, we decided to use



IV-6

the McQuillin approach to the synthesis of the cyperones[97] in our attempt to make synthetic (IV-1).

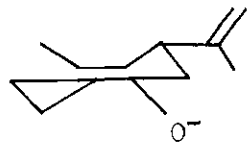
McQuillin has utilized the Robinson annelation procedure to prepare ketol (IV-7) in good yield.[97] The reaction simply involves the condensation of the quaternary ammonium salt of ethyl vinyl ketone with the enolate of dihydrocarvone (formed from the addition of dihydro-



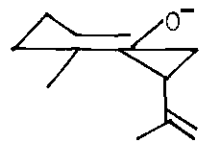
IV-7

carvone to a stirred suspension of $NaNH_2$ in ether) in the presence of pyridine. The absolute configuration of the crystalline ketol product

is determined by the absolute configuration of the dihydrocarvone used. l-Dihydrocarvone gives the absolute configuration indicated above, whereas d-dihydrocarvone gives the mirror image, which is not the natural configuration. The relative stereochemistry of the product has been discussed by McQuillin[97] and others.[109] The Δ1 enolate of dihydrocarvone can exist in two different conformations (IV-8) and (IV-9) which differ

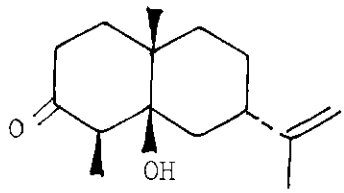


IV-8

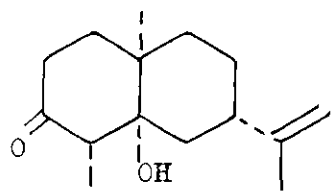


IV-9

in the orientation of the isopropenyl groups. Enolate (IV-8) should be more stable in that the isopropenyl group is equatorially oriented. Addition of EVK then occurs from the side most remote from the isopropenyl group in enolate (IV-9) which would give ketol (IV-11). Addition of EVK to (IV-8) occurs from the bottom side so that a chair intermediate can form, giving ketol (IV-10). Since (IV-8) is the pre-

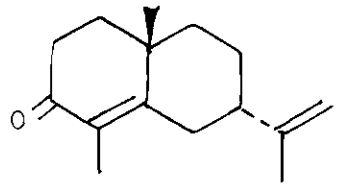


IV-10

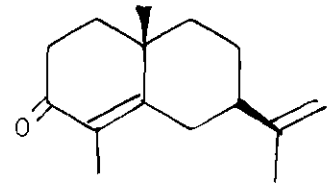


IV-11

dominant enolate, (IV-10) is expected to be the major product, which it is. Fortunately, (IV-10) crystallizes readily from the reaction mixture, hence purification is simple. McQuillin also found that the non-crystalline portion of the reaction mixture was mostly a mixture of dehydrated isopropenyl epimers (IV-12) and (IV-13).



IV-12



IV-13

Our synthesis started with the classic McQuillin procedure.[97] Ketol (IV-10) was prepared, as its enantiomer, as discussed above, but the crystalline ketol was not isolated. Since we had planned to invert the isopropyl stereochemistry shortly, it was decided to use the mixture containing (IV-10), (IV-12) and (IV-13) as is. These products were collected by vacuum distillation of the reaction mixture on a 36 inch stainless steel spinning band column; they boiled in the range of 84° to 92° at 0.15 mm. Our sequence started with d-dihydrocarvone, hence we synthesized the enantiomers of all the natural products mentioned. It should be mentioned that in recent years several more efficient methods of preparing (IV-10) have been reported.[110,111]

Once the bicyclic structure was established, our synthesis plan could take form. The following transformations must take place to yield the target molecule:

(1) The stereochemistry of the isopropyl group must be inverted.

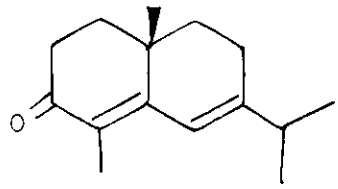
(2) A trans ring junction must be introduced.

(3) A carbonyl group must be placed at C-1.

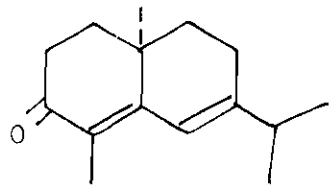
(4) An exocyclic double bond must be introduced at C-4.

Development of an efficient synthesis to achieve these goals has been realized.

Looking at the target molecule, we immediately see that the first two goals should be easily achieved. The stereochemistry of the isopropyl group in the final product is the thermodynamically most stable conformation (in fact, this is why Bhattacharyya assigned it that stereochemistry) and also the trans ring junction of the decalones with C-6 equatorial substituents is the most stable form.[112] Turning back to McQuillin's work, we see that dehydration of ketol (IV-10) in 50 percent v/v sulfuric acid - water gives (+)β-cyperone (IV-14) in good yield.[97] Since our first two goals involve the formation of the thermo-

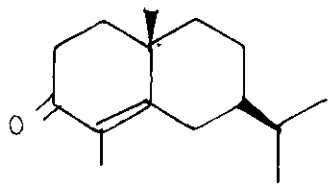


IV-14

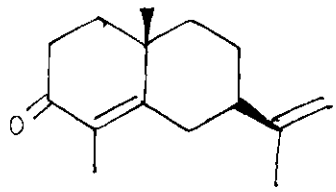


IV-15

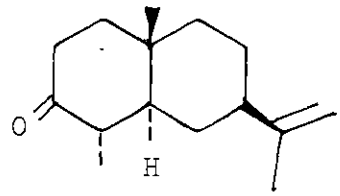
dynamically most stable product, chemical reduction of β-cyperone should be the approach of choice.[113] Howe and McQuillin have studied the course of reduction of the cyperones.[100] Chemical reduction of (IV-14) using zinc in acetic acid gave (IV-16) in which the isopropyl group assumes



IV-16



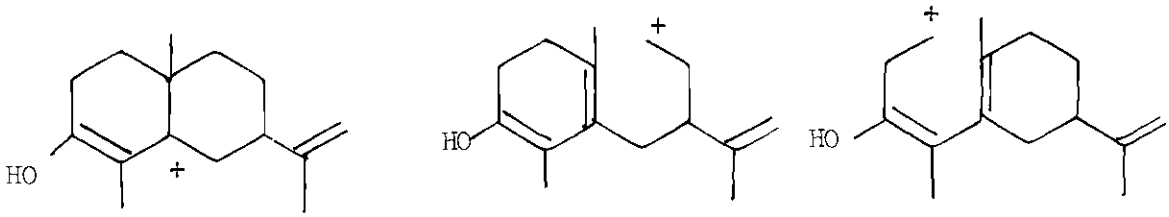
IV-17



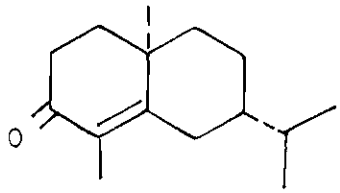
IV-18

the most stable orientation (β-equatorial). Partial catalytic hydrogenation gave the same product. Similarly, reduction of (+)α-cyperone (IV-17) with lithium in ammonia gave the most stable <u>trans</u> ring junction (IV-18).

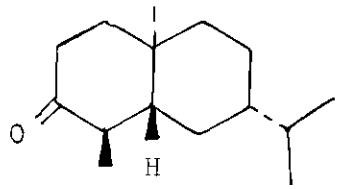
Preparation of β-cyperone (as its enantiomer, (IV-15)) proceeded in good yield when the mixture of ketol and dehydrated material was used ((IV-10), (IV-12) and (IV-13)). The spectral properties are consistent with those reported by McQuillin[97] and others.[98] Optical rotation measurements at the mercury green line (546.1 mμ) indicated an optical purity of greater than 50 percent (57 percent) which is reasonable in that optically impure material was used in the dehydration step. McQuillin has reported that racemization occurs during dehydration possibly through the following intermediates:[97]



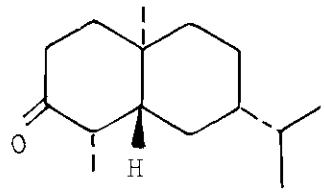
After (-)β-cyperone had been prepared, it was decided to attempt a chemical reduction of both double bonds in one step. Reduction of (-)β-cyperone with lithium in ammonia using anhydrous ether and anhydrous ethanol as co-solvents did not give the desired tetrahydro-product, but did give an excellent yield of (-) dihydro-α-cyperone (IV-19).



IV-19



IV-20



IV-21

Again, spectral properties were similar to those reported.[99] Optical purity was still greater than 50 percent (52 percent). The decrease in optical purity could, in part, be due to the fact that a concentration of 1.5 grams per 100 ml was used for this measurement, while the reported measurement was taken at a concentration of 4.8 grams per 100 ml. It shall be assumed that the compounds synthesized throughout this sequence are in about 50 percent optical purity, since no further reaction will be done which can affect the isopropyl or C-10 methyl configurations.

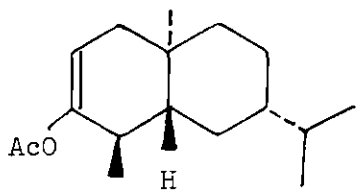
Chemical reduction of (IV-19) using lithium in ammonia, with ether as a co-solvent and later ammonium chloride as a proton source, gave (IV-20) as the major product (90 percent). It is believed that (IV-21) is produced along with (IV-20) in small (approximately 10

percent) amounts. This compound could be detected as a shoulder when certain VPC columns were used (Col. II, III, IV and V - see Experimental section) to check purity. No column, at the lengths used, would give a clean separation of the two compounds. On open column chromatography the methyl epimers behaved identically. Further arguments shall be presented later to fully establish the structure of the contaminant. Since the stereochemistry at C-4 will be destroyed later when an exocyclic methylene group is created, the Birch product was used as is.

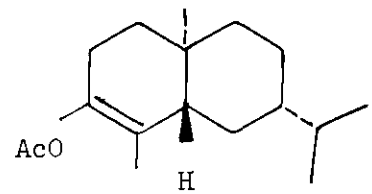
We have, with the above steps, completed the first two goals of our synthesis scheme in a relatively simple and efficient fashion. Since all the compounds made had been prepared previously, our work was made a little easier. It is felt that this sequence is much simpler than those previously used, and when coupled with the ketol preparation procedures developed by Hortmann and co-workers,[111] results in an extremely efficient synthesis (approximately 30 percent overall yield from ethylene to the eudesmane skeleton). Modifications in ordering of reactions could result in functionality which would readily lead to other eudesmane sesquiterpenes.

The third step of our synthesis, introduction of oxygen at C-1, was successfully accomplished through the Δ2 enol acetate of (IV-20). Bromination of this compound led, after dehydrohalogenation, to the α,β-unsaturated compound (IV-25) which was epoxidized. Wharton rearrangement[114] led to the allylic alcohol (IV-27). Oxidation of this compound gave the desired ketone at C-1. Let us now discuss these steps in more detail.

Preliminary studies had shown that direct bromination and dehydrohalogenation of (IV-20) was not an efficient route to the desired α,β-unsaturated ketone.[115] It was found that enol acylation using isopropenyl acetate as the solvent and p-toluenesulfonic acid as a catalyst, with removal of the acetone formed, gave a good yield of the desired Δ2 enol acetate (IV-22). A preliminary experiment using a heating mantle as a source of heat gave, after 24 hours, a product composition which



IV-22



IV-23

was approximately 80 percent desired enol acetate (IV-22) and 20 percent undesired enol acetate (IV-23). House and Trost have studied the formation of enol acetates under these conditions and have stated that the less substituted isomer forms more readily.[116] In a later experiment, run under identical conditions, a product composition of 60 percent (IV-22) and 40 percent (IV-23) was realized. This product ratio was deemed unsatisfactory for further synthetic work. Attempts to separate the two isomers failed and it was decided to study the reaction further, so that the original product composition could be made reproducible. Hydrolysis of the enol acetate was accomplished by stirring it overnight in a solution of 10 percent methanolic HCl. Milder hydrolysis conditions failed to effect any reaction.

The ketone (IV-20) was then re-reacted as before, but this time the reaction was carefully followed by VPC. The results are summarized in Table 4. These results are graphically displayed in Figure 4.

Table 4. Enol Acylation of (IV-20).

Time (hrs)	% (IV-20)	% (IV-22)	% (IV-23)
1.0	36.5	50.5	13.0
1.5	33.0	51.0	16.0
2.0	31.0	51.5	16.5
3.0	25.0	53.0	21.0
3.5	23.0	54.0	23.0
4.0	21.0	56.0	24.0
4.5	19.0	56.0	25.0
5.0	17.0	57.0	27.0
5.5	15.5	57.5	27.0
24.0	0.0	60.0	40.0

It is felt that these results support the observation of House and Trost.[116] Clearly the Δ2 (less substituted) enol acetate is formed first and the Δ3 (more substituted) enol acetate is formed slower. Obviously, the Δ2 enol acetate is the kinetic product and the Δ3 enol acetate is the thermodynamic product. Since the reaction was never conducted for longer than 24 hours, it is not known whether further conversion of the Δ2 enol acetate to the Δ3 enol acetate would occur. The assignments of

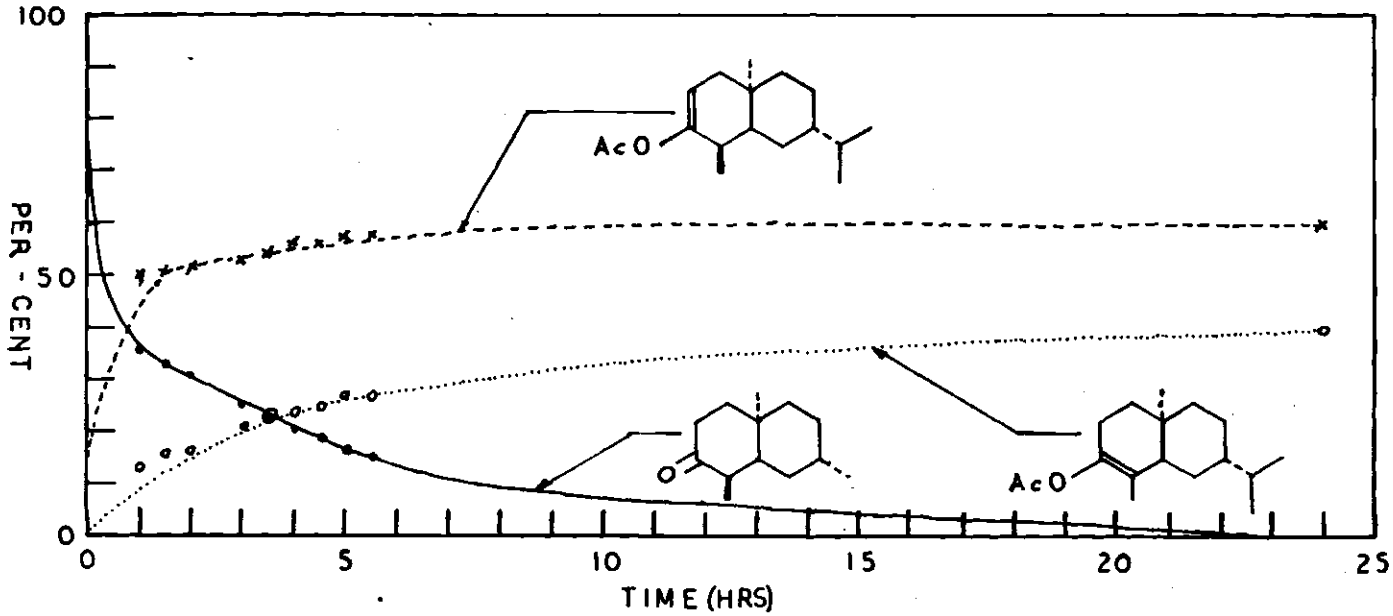
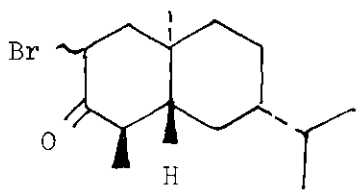


Figure 4. Enol Acylation of (IV-20).

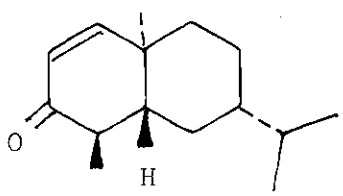
VPC retention times were based on an analysis of the NMR spectrum of the final product composition, which clearly indicated a 2:3 product ratio of Δ2 to Δ3 enol acetates. From this, the peak in the VPC with the smaller area was the one representing the Δ3 enol acetate.

Since a product ratio of 4:1 was obtained previously using a heating mantle as a source of heat, it was decided that the only experimental difference between the reactions was the rate of reflux. While the temperature of the two reactions would be the same, the amount of energy (heat) available would be greater in the reaction undergoing more vigorous reflux. To control this variable, a temperature-controlled oil bath at 110° was used as a source of heat. As expected, a 4:1 product ratio was realized. Repetition of this reaction yielded a very similar product ratio. When the oil bath was used, approximately 4 percent starting ketone remained after 24 hours, indicating that this simple trick had indeed slowed down the reaction so that a synthetically useful product yield could be obtained. This product was further reacted without additional purification.

Bromination was readily accomplished using molecular bromine dissolved in CCl_4. Epichlorohydrin was used to absorb the H^+ formed.[117] The bromide (IV-24) was not purified, but reacted as recovered. Dehydration to the α,β-unsaturated ketone (IV-25) was accomplished using



IV-24



IV-25

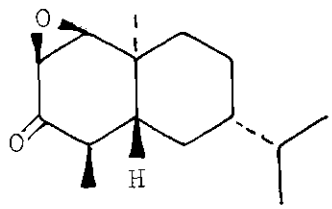
LiCl and Li_2CO_3 in dimethylformamide (DMF). A preliminary reaction showed that the formation of a black solution occurred. Attempts to obtain a cleaner looking reaction mixture failed, and under all conditions (N_2 atmosphere, anhydrous reagents and solvent, mild heat) a black reaction was obtained. The DMF and volatile eudesmane products were separated from the black polymer-like material by fractional vacuum distillation. Approximately 90 percent (by weight) of eudesmane products were isolated. This material contained 43 percent of the desired product (IV-25), 23 percent of (IV-19) formed from the Δ3 enol acetate, and 15 percent of starting ketone (IV-20), part of which remained during the enol-acylation reaction and part of which probably came from subsequent, unwanted hydrolysis. A product making up 11 percent of the eudesmane material was observed and was not identified, but was probably unreacted enol acetate.

The reaction products could be separated by chromatography on activity II neutral alumina and all had roughly the same chromatographic properties. A fraction containing a 1:1 mixture of starting ketone (IV-20) and desired product (IV-25) was obtained in 30 percent ether - pet ether, and pure (IV-25) was isolated in the subsequent 35 percent ether - pet ether fraction. Spectral properties were characteristic of the assigned structure. In particular the low field AB system in the NMR spectrum was very characteristic of the isolated olefinic protons.[105] No methyl on a double bond was observed in the NMR. The mass spectrum was distinctly different from the isomeric (IV-19). Combustion analysis was correct for the assigned formula. ORD-CD-UV

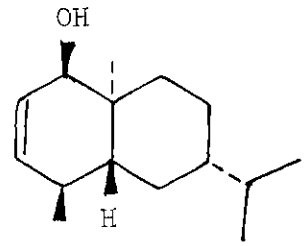
spectra were characteristic of an α,β-unsaturated ketone. A multiple Cotton effect curve due to the n → π* transition was clearly evident both in MeOH and dioxane.[118]

Overall yield (isolated and pure) from the starting ketone (IV-20) was 21 percent. Since the sequence involves both the 50 percent dehydration reaction and selective formation of one enol acetate, it is felt that the 21 percent yield over the three steps was good. However, House and co-workers have recently reported procedures for selectively forming the less substituted enol acetate of 2-methylcyclohexanone in nearly quantitative yield (99 percent) using lithium diisopropylamide as a base, adding the ketone to excess base, and quenching with acetic anhydride.[108] It is felt that any reinvestigation of this sequence should involve the study of the use of this reaction either to form the enol acetate or to directly form the bromide. A good yield of the desired enol acetate would improve the overall yield somewhat, but more importantly, should simplify the final purification steps.

Movement of the oxygen functionality from C-3 to C-1 was readily accomplished in two steps. Epoxidation of (IV-25) was performed by reaction with basic peroxide. VPC analysis showed one predominant peak which was the epoxide (IV-26). Only 10 percent starting material remained; however, attempts to purify it by open column chromatography techniques led to little enhancement of its purity. Mechanistically, attack should come from the least hindered side, giving the stereochemistry shown.[119] No evidence for the formation of the other epoxide



IV-26



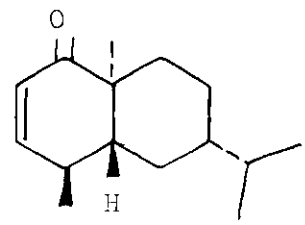
IV-27

epimer could be found. Infrared spectra showed loss of the conjugated ketone and formation of an epoxy-ketone at 1725 cm^{-1}.[105] The NMR spectrum showed no starting material and showed a doublet of doublets at δ3.23 and 3.35 characteristic of the H-1 and H-2 coupling. While the purity was deemed slightly less than that required for combustion analysis, an exact mass determination of the m/e 236 peak clearly established the proposed molecular formula.

Wharton has developed a method for rearranging α,β-epoxy-ketones to allylic alcohols with the hydroxyl group at the former β position, using hydrazine in ethanol containing a trace of acetic acid.[114] Mechanistically, not much is known about this reaction; however, it is felt that the stereochemistry of the β oxygen bond should be retained. Wharton rearrangement of (IV-26) gave (IV-27) as the only VPC volatile product. When impure epoxide, containing either α,β-unsaturated ketone (IV-25) or saturated ketone (IV-20), was reacted under Wharton conditions, only one peak could be observed on the VPC which had a retention time different from the other compounds. It is not known whether the impurities formed hydrazones that were not volatile, formed hydrazones which came off under the solvent peak, or

whether reduction to the hydrocarbon occurred (an unlikely event). Spectral properties again fit the assigned structure. No carbonyl absorption was evident in the infrared spectrum, but absorptions due to hydroxyl and a cis disubstituted double bond were present. NMR analysis showed one exchangeable hydrogen at δ3.72 and a complex absorption at δ5.47-6.00 due to the vinyl protons. Exact mass determination clearly established the molecular formula.

Now that the oxygen has been placed at C-1, one must convert it to the desired ketone functionality. Allylic alcohols are supposedly oxidized to α,β-unsaturated ketones by MnO_2.[120] Preparation of activated MnO_2 by the Attenburrow procedure[101] and reaction with allylic alcohol (IV-27) gave only a poor yield of the desired product. It is felt that this was due to the difficulties involved with recovering all the material from the MnO_2; hence, this method was deemed a poor synthetic approach. Alcohols are smoothly oxidized to ketones by Jones reagent[46] and this method was tried on the allylic alcohol. Reaction of (IV-27) with Jones reagent gave α,β-unsaturated ketone (IV-28) in excellent yield (85 to 95 percent in repeated experiments).



IV-28

VPC analysis of this product showed it to be pure on five columns. On columns which could separate the two C-4 epimers, which

were formed in the earlier Birch reduction step, 10 percent of the axial isomer was observed. The loss of hydroxyl absorption was noted in the infrared and the formation of an α,β-unsaturated ketone was evident. The NMR showed a low field ABX system characteristic of the structure.[105] There was no significant difference in the NMR absorption of the angular methyl protons between the isomeric α,β-unsaturated ketone (IV-25) and (IV-28) (δ1.03 versus δ1.00). The mass spectra of the two were different, (IV-25) having a base peak at m/e = 174 and (IV-28) having one at m/e = 82. The ORD-CD of (IV-28) showed a multiple Cotton effect in both methanol and dioxane for the $n \rightarrow \pi^*$ transition, characteristic of the α,β-unsaturated ketone chromophore.[118] The $\pi \rightarrow \pi^*$ transition showed the usual anomalous Cotton effect curve whose (+) sign was characteristic of the helical nature of the chromophore.[118] Combustion analysis clearly established the assigned molecular formula. Preparation of this compound fulfills step three of the synthetic plan.

All that is left is the generation of an exocyclic double bond at C-4 and elimination of the Δ2 double bond. Functionality could be introduced at C-4 by virtue of its being the γ position of α,β-unsaturated ketone, making its proton more acidic than the others on the molecule.[121] Also, the position is allylic, making it a likely position for free radical attack. Attempts to utilize these properties were made. Several other methods were also tried. Let us now look at these methods.

One approach to this synthesis would be the addition of atomic

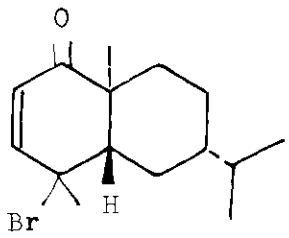
bromine to C-4 through the use of N-bromosuccinimide (NBS) as a reagent. Eaton and Cole have added atomic bromine to the γ position of an α,β-unsaturated ketone in their successful synthesis of cubane.[103] Reaction of NBS with (IV-28) for five minutes, in CCl_4 at room temperature under ultraviolet light, gave no reaction. Reaction for two hours at room temperature using a 250 watt Hanovia lamp for an energy source gave one major product, and a lot of minor products. The starting ketone was stable to irradiation from the lamp for one hour when no NBS was added. Chromatography of the mixture showed that most of the material was polymeric in nature, and the major peak had no carbonyl absorption in the infrared spectrum. This method was not investigated further.

It was decided that the use of an external light source, other than room lighting, was causing unwanted side reactions. The NBS and ketone were dissolved in CCl_4 and refluxed.[104] Observation of the reaction by VPC showed no reaction after five minutes. After one hour a peak with a retention time similar to starting material had appeared. After two hours, the reaction was stopped and an IR and NMR were taken. The IR showed the presence of a non-conjugated ketone and the NMR showed the presence of a methyl on a double bond. Starting material was evident in both spectra. Also the NMR appeared to have absorptions due to more than two compounds.

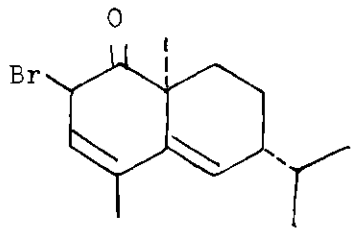
Since starting material was still present it was decided that the reaction should be continued. After six hours no further change was noted in the VPC; however, the reaction was allowed to continue until 10.5 hours total reaction time had elapsed. A thin layer

chromatograph of the worked up reaction product showed that at least eight compounds were present, which were separated by preparative thin layer chromatography. No compound was present in greater than 10 percent yield, hence the reaction was deemed synthetically useless. However, it was desirable to achieve some idea about what was happening in this reaction.

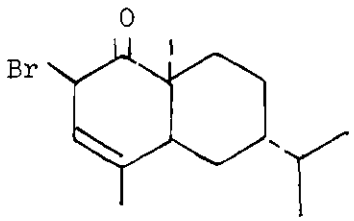
Infrared and mass spectra of each fraction were taken. No fraction which had a conjugated ketone in the IR showed bromine in the mass spectrum, hence none of the desired bromide (IV-29) was present. Several fractions showed non-conjugated carbonyls and bromine in the



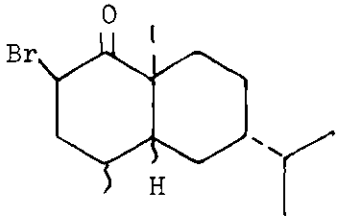
IV-29



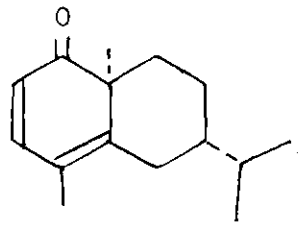
IV-30
($C_{15}H_{21}OBr$)



IV-31
($C_{15}H_{23}OBr$)



IV-32
($C_{15}H_{25}OBr$)

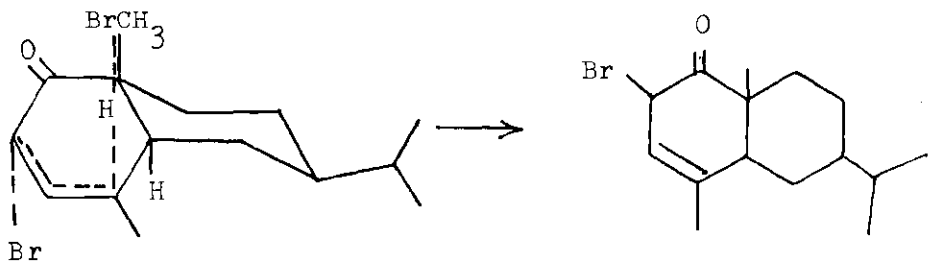


IV-33
($C_{15}H_{22}O$)

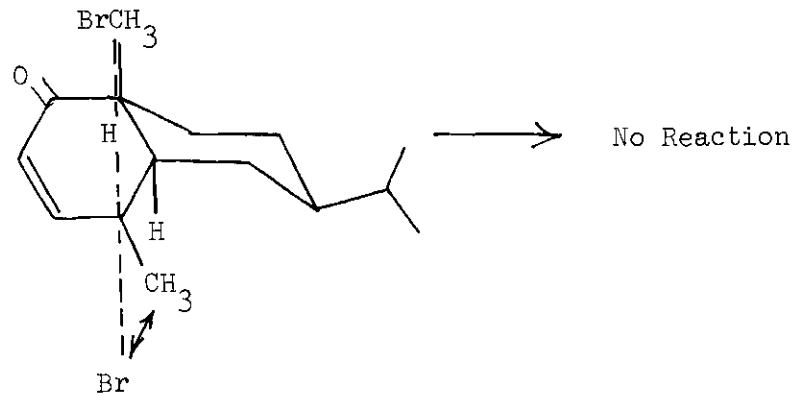
mass spectrum. These showed M^+'s of 297 ($C_{15}H_{21}OBr$), 299 ($C_{15}H_{23}OBr$)

and 301 ($C_{15}H_{25}OBr$). A fraction with an M^+ of 218 ($C_{15}H_{22}O$) and having a conjugated ketone in the IR was observed. One can assign rational structures (IV-30) through (IV-33) to these compounds.

It is felt that these anomalous results are due to a steric inaccessibility at C-4, caused in part by it being a tertiary center and in part by the C-10 angular methyl group. When atomic bromine attacks at this position the proton is only slowly removed. The C-10 angular methyl allows attack only from one side of the molecule; if a true free radical is formed at C-4 no problem would arise. However, if the radical is not truly free, but has bromine near, then another atom of atomic bromine must add from the same side as the non-planar C-4 methyl group. Addition at C-4 would then be unfavorable because of the steric hindrance. However, addition at C-2, which is the other end of the allylic radical, would be more favorable sterically (see below):



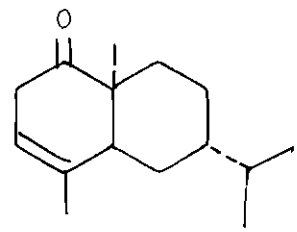
IV-31



Electronically, C-2 is the more favorable end of the allylic system since it is also allylic to the carbonyl. The other unwanted products are probably due to side reactions occurring during the long reaction time. If (IV-30) is formed, it is only reasonable to suspect (IV-32) would be present. It should be mentioned that evidence for these structures is weak (only an IR and mass spectrum) but the mechanism mentioned would easily explain the observation of a non-conjugated ketone and a methyl on a double bond in the NMR after two hours. Also, the presence of other olefinic protons of small intensities in the NMR can be explained by this mechanism. Two results are clear from this study. They are that the scope of the NBS reagent has not been fully explored, and that this reaction is synthetically useless for our purposes.

Since we could not make use of the allylic nature of C-4, it was decided to try to utilize the acid nature of its proton.[121] Basic deconjugation of α,β-unsaturated ketones has been utilized in steroid[107] and sesquiterpene synthesis.[122] The approach involves total conversion

of the ketone to its enolate and then rapid quenching of the enolate with water or dilute acetic acid. Kinetic quenching protonates exclusively α to the carbonyl, since it is the electron rich position, and a deconjugated product is formed. Standard conditions are t-BuOK in t-BuOH with a reaction time of one to two hours. Use of these conditions on (IV-28) failed to yield any reaction. When a reaction time of three days was utilized, an impure yield of approximately 20 percent deconjugated ketone (IV-34) was realized. Conducting the reaction at higher temperatures did not improve the yield. Use of

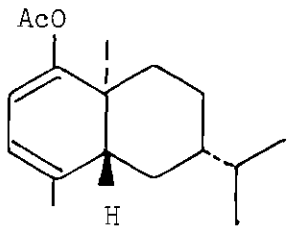


IV-34

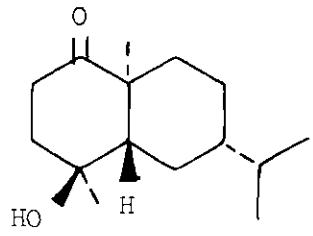
sodium hydride in dioxane or trityl lithium in benzene did not give any carbonyl product. In all cases, polymeric material was observed visually and spectrally. The low yields and unwanted side reactions caused by long reaction times caused us to investigate the utility of the amide base techniques recently developed by House and co-workers.[108] Reaction of (IV-28) with lithium n-propylamide, lithium diethylamide and lithium diisopropylamide failed to give any detectable reaction. Short reaction times were used since the previous studies with both NBS and other bases had shown that side reactions predominate as the reaction time is increased. It is felt that the problem involved

with base deconjugation is simply the steric inaccessibility of the C-4 position, caused by its being a tertiary center and by the C-10 angular methyl, which is on the same side as the proton we wish to abstract. The enolate is hence formed so slowly that there is always ketone available for it to react with, forming aldol-type condensation polymers. With short reaction times, as with the amide bases, no significant concentration of enolate was formed, hence no detectable product was formed. Again it was decided that this method was synthetically useless in our case.

An exotic approach was then discussed. It was felt that the addition of singlet oxygen[27d] to enol acetate (IV-34) followed by reduction would yield ketol (IV-35) which could then undergo elimin-



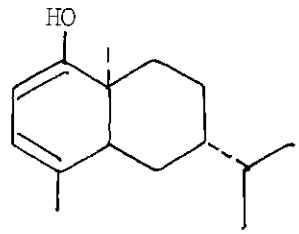
IV-34



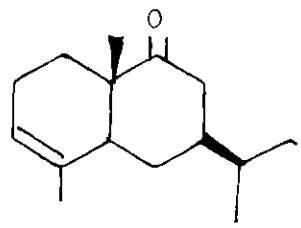
IV-35

ation to give the target molecule. Unfortunately, attempts to form the enol acetate were unsuccessful. Reaction of (IV-28) with isopropenyl acetate, with trace amounts of p-toluenesulfonic acid, gave no enol acetate product. Reaction with acetic anhydride in CCl_4 using $HClO_4$ as a catalyst showed, in the VPC, a reasonable rate of disappearance of the ketone, but no appearance of any new peaks. IR and

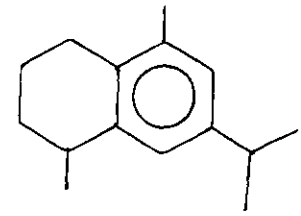
NMR showed no enol acetate was formed. These results were very unexpected, so it was decided to see what happened to (IV-28) when it was placed in a solution containing a trace amount of $HClO_4$. A solution of (IV-28) was prepared in an NMR tube and its spectrum was taken. A trace amount of $HClO_4$ was added and the solution immediately turned black. After 1.25 hours, a broad peak at δ7.67 appeared and some change in the methyl region was noted. The olefinic protons were in the same position and of the same intensity as at the start. The broad peak could be due to the enol (IV-36). After 2.25 hours, the peak at δ7.67 disappeared and changes were again



IV-36



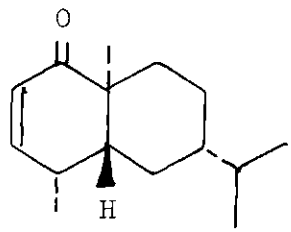
IV-37



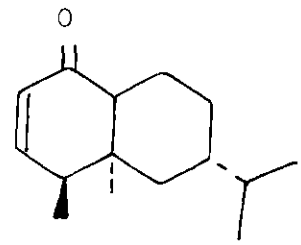
IV-38

noted in the methyl region. The α,β-unsaturated ketone system of (IV-28) was unchanged. Changes were continually noted in the methyl region up till six hours, when observation was stopped.

Under Lewis acid conditions, Zalkow and Lacoume had noted that (IV-37) gave (IV-38).[95] It is clear that under acidic conditions (IV-28) does not give an analogous aromatic system. Since the α,β-unsaturated ketone structure remained unchanged, but the methyl region had changed, it is felt that a methyl rearrangement of some type had occurred. One possibility is that the C-4 methyl had epimerized to give (IV-39). Methyl rearrangement to (IV-40) could



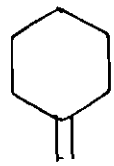
IV-39



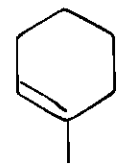
IV-40

be a possibility. While this would be a significant achievement, hard evidence for it is lacking, and it would be very premature to claim that this has been accomplished. While our NMR study was interesting, it did not tell us why no enol acetate was formed and we are still at a loss to explain it.

We next turned to the recent work of Marshall,[123] expanding on preliminary studies of Kropp and Krauss,[102] involving the movement of an endocyclic double bond exocyclic by using light. Kropp and Krauss obtained excellent yields of (IV-41) from (IV-42) by using Vycor-filtered Hg radiation from a 450 watt Hanovia lamp, in a protic solvent containing an aromatic sensitizer. Their work showed that



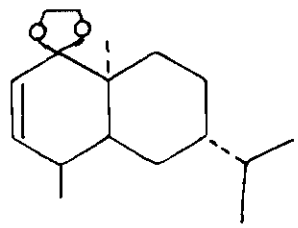
IV-41



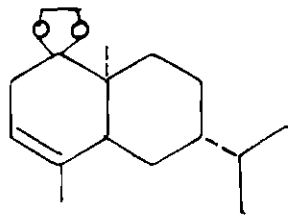
IV-42

benzyl alcohol was ideal for this reaction. It was decided to try this method on our ketone intermediate (IV-28). In an attempt to prevent unwanted side reactions, the ketal of (IV-28) was formed.

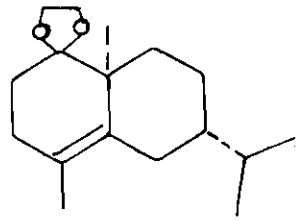
Reaction of (IV-28) with ethylene glycol in benzene, with p-toluenesulfonic acid as a catalyst, and removal of the water, gave an excellent yield of three ketals. NMR analysis of the mixture



IV-43



IV-44

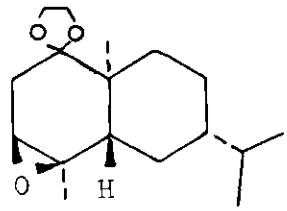


IV-45

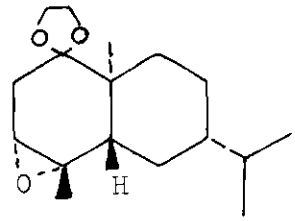
showed the presence of (IV-43), (IV-44) and (IV-45) in a 1:3.4:3.7 ratio. Combustion analysis of the mixture showed that the formula $C_{17}H_{28}O_2$ was correct. This mixture was irradiated under the conditions of Kropp and Krauss using both Pyrex and quartz-filtered Hg radiation from a 450 watt Hanovia lamp (a Vycor apparatus was not available). In all cases polymeric material was clearly evident (white polymer when MeOH at 0° was used as a lamp coolant, brown polymer when water at approximately 20° was the coolant). Chromatography over 10 percent $AgNO_3$ - silica gel gave (IV-44) and (IV-45) as the only isolated products. No exocyclic material was noted.

Since base was not useful for the deconjugation of the ketone and since deconjugated material was observed from the ketalization reaction, it was decided to approach the target molecule through the ketal. The $\Delta 3$ double bond ketal (IV-44) was isolated from the ketal mixture by chromatography over 10 percent $AgNO_3$ - silica gel. NMR supported the structure for this compound in that four ketal

protons, one olefinic proton and three olefinic methyl protons were observed. Epoxidation of this molecule was smoothly accomplished by stirring it overnight with m-chloroperbenzoic acid in dichloroethane as a solvent. VPC analysis revealed two epoxides in a 1:3.5 ratio. Since epoxidation occurs predominantly from the least hindered side with this reagent,[124] the major product was thought to be the α-epoxide (IV-46) and the minor product was the β-epoxide



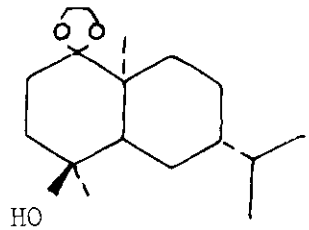
IV-46



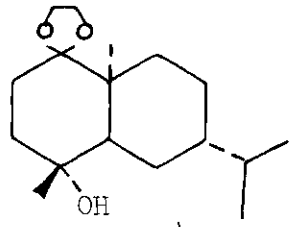
IV-47

(IV-47). The NMR supported the assigned structure in that the C-3 proton appeared as a clean triplet in the region expected. A good deal of complexity was observed in the region of the NMR where the ketal protons absorb. It is felt that several different ketals were present in the solution ((IV-46), (IV-47) and some (IV-44)) with each ketal absorbing slightly differently, causing the complexity.

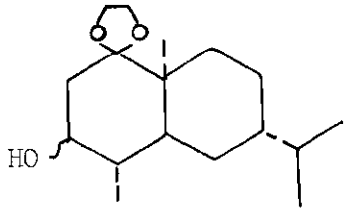
The epoxide mixture was smoothly opened to the alcohol using lithium aluminum hydride. Mechanistically, the reduction gives the most substituted alcohol, with the stereochemistry of the epoxide C-O bond retained.[125] Hence the major product should be (IV-48). However, the reaction is not that stereospecific, so some (IV-50) was expected. Since (IV-47) was a contaminant, (IV-49) and (IV-51) should



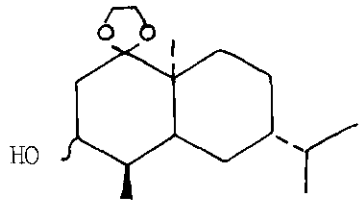
IV-48



IV-49



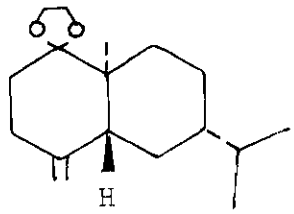
IV-50



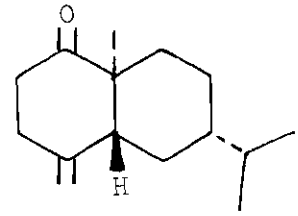
IV-51

also be present. As was expected, the VPC of the reaction product showed more than one or two peaks. Unfortunately, the retention times were similar to the starting epoxide and no analysis of the product ratio could be undertaken. The NMR showed none of the characteristic C-3 proton triplet of the epoxide, so that the epoxide was present, if at all, in less than 5 percent amounts. An exchangeable proton was observed in the NMR, as was a hydroxyl absorption in the IR. Again the ketal proton region of the NMR was quite complex due to the multitude of products present.

Dehydration of the alcohol mixture was done as before,[95] using $POCl_3$ in pyridine, and the exocyclic ketal (IV-52) was observed to be the major product by VPC. Some starting alcohol remained. No $\Delta 3$ double bond ketal (IV-44) was observed in the VPC; however, there is a strong possibility that (IV-43) was also formed. The exocyclic



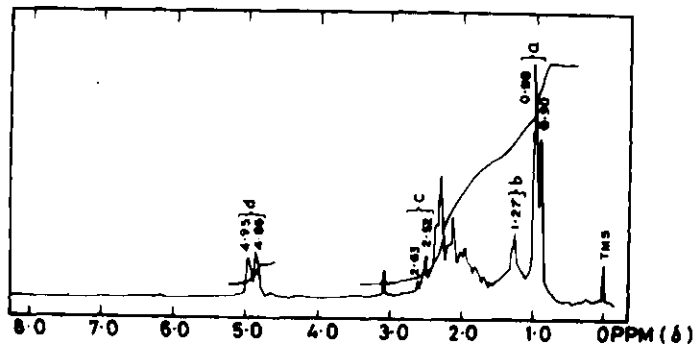
IV-52



IV-53

ketal was then isolated by chromatography on 10 percent $AgNO_3$ - silica gel and hydrolyzed under very mild conditions to yield (IV-53) in 80 percent purity. An NMR of this material is shown in Figure 5c. Chromatography of this sample as before yielded pure (IV-53) in 2 percent overall yield from (IV-28). The low yield was due mostly to the problem of total recovery of material that was noted whenever a silver nitrate column was used. VPC data indicated that the conversion was actually more efficient.

The NMR spectrum of (IV-53) is shown in Figure 5b. Unfortunately, the spectrum is not well resolved due to the small size of the sample. However, it faithfully reproduces the features of the 80 percent pure spectrum, which is of much better resolution. Reproduced in Figure 5a is the NMR spectrum of natural canarone as kindly supplied by Dr. Bhattacharyya. Also shown in Figure 6a and Figure 6b are the infrared spectra of natural canarone and synthetic (IV-53). In Figure 7a and Figure 7b are the ORD curve of natural canarone[24] and the ORD-CD-UV curves for synthetic (IV-53). As pointed out earlier, the optical purity of this, and all previous synthetic material, is taken to be about 50 percent. Comments concerning the structure of

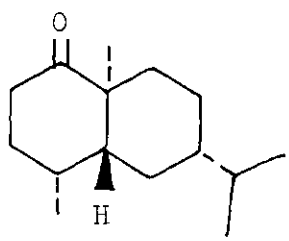


NMR SPECTRUM OF THE CANARONE IN
CCl_4 / TMS, VARIAN 60 Mc.

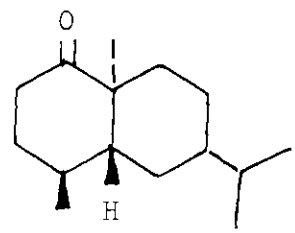
Figure 5a. NMR Spectrum of the Canarone in CCl_4/TMS, Varian 60 Mc.

canarone as related to (IV-53) shall be reserved until later.

It was also deemed useful to have complete spectral data on the dihydroderivative of (IV-53) and its C-4 methyl epimer. Since only limited quantities of (IV-53) were available, the dihydroderivative was prepared by hydrogenation of (IV-44). Mechanistically, hydrogenation should occur from the α face of both molecules due to the steric bulk of the C-10 angular methyl group.[126] If structure (IV-1) is correct for canarone, then this product (IV-54) should be the enantiomer of dihydrocanarone. We shall comment later on the spectral properties of (IV-54) versus those reported for dihydro-



IV-54



IV-55

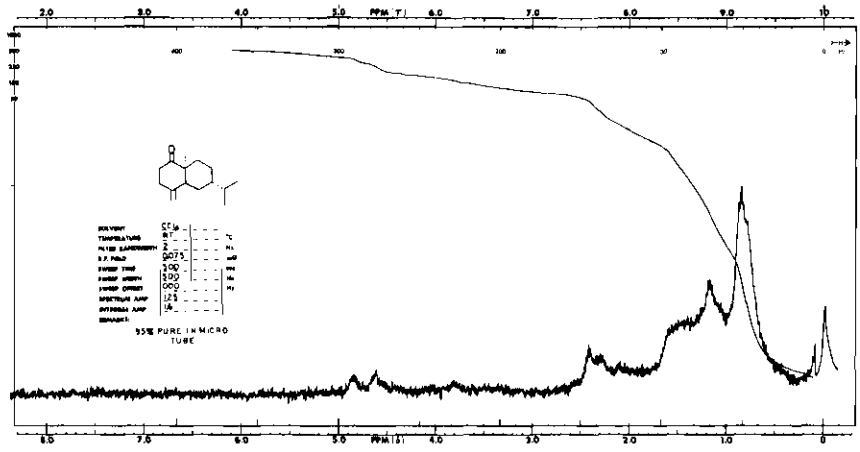


Figure 5b. NMR of Pure (IV-53).

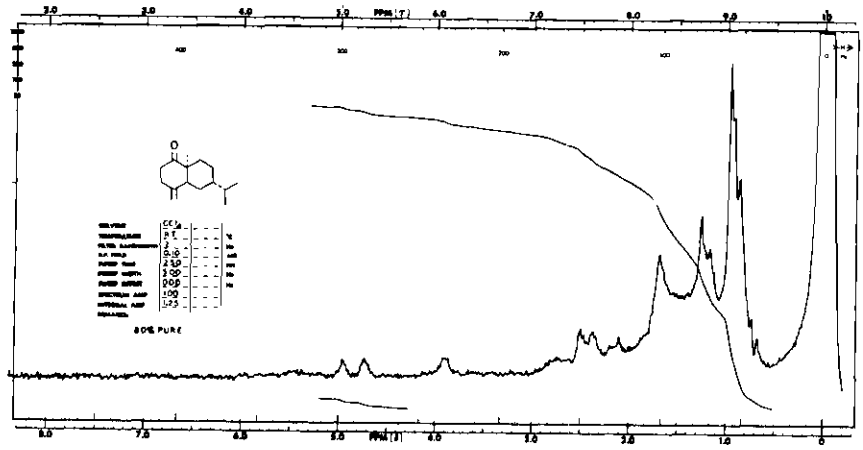


Figure 5c. NMR of 80 Percent Pure (IV-53).

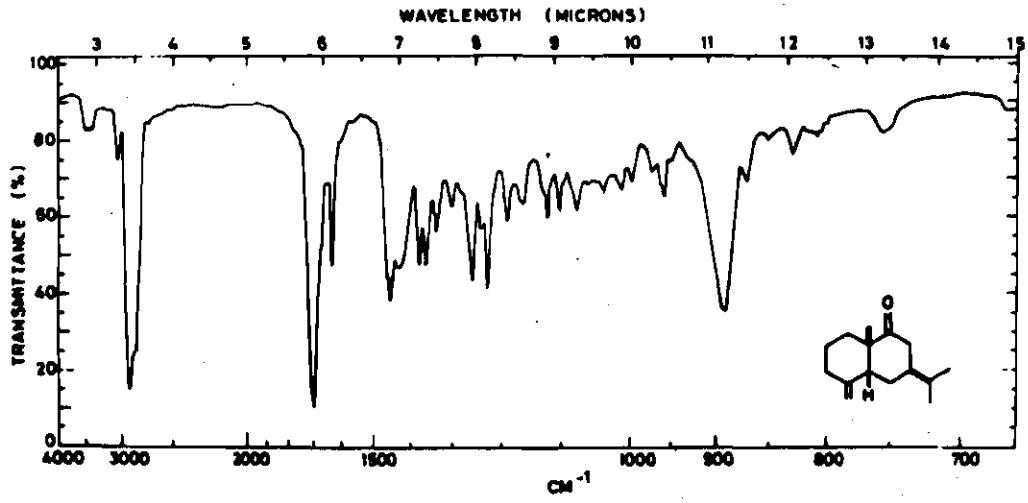


Figure 6a. IR Spectrum of the Canarone from Black Dammar Resin.

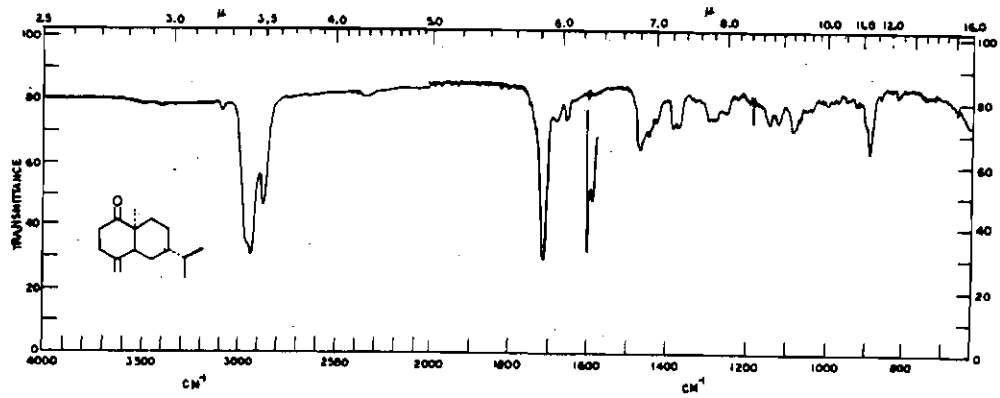


Figure 6b. IR of Pure (IV-53).

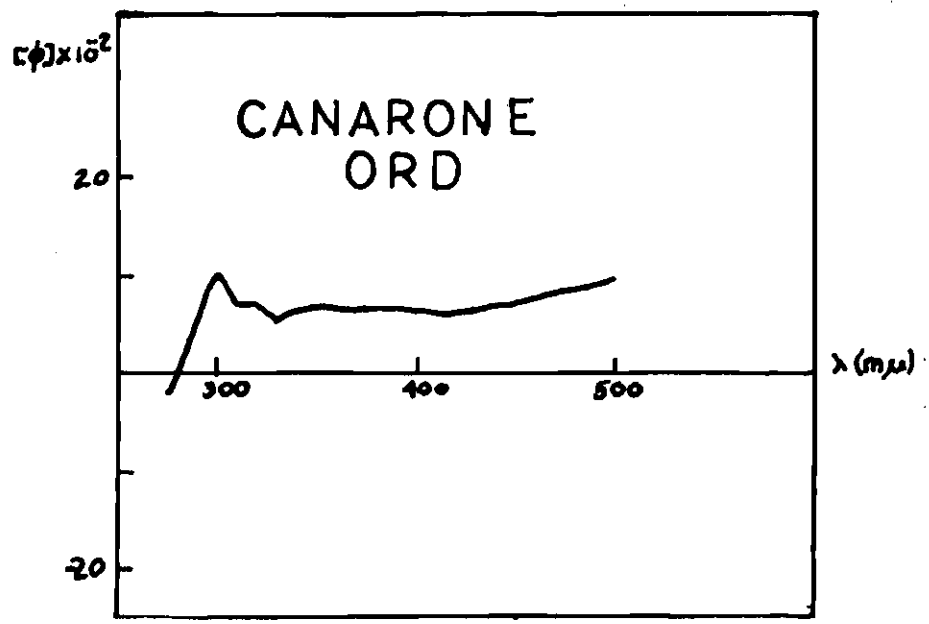


Figure 7a. Published ORD of Canarone[24].

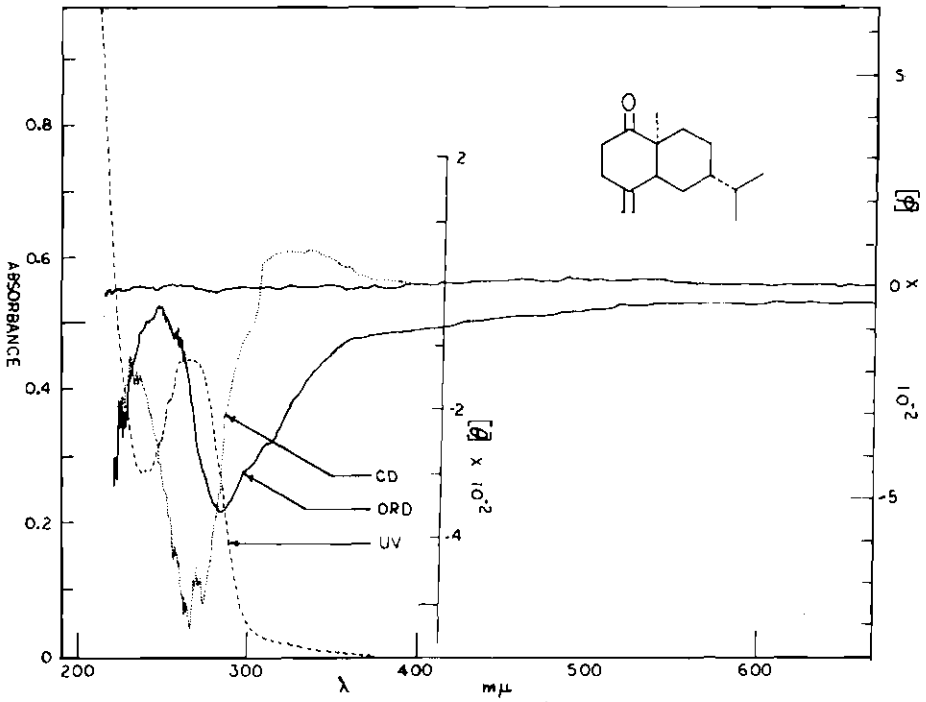


Figure 7b. ORD-CD-UV of Pure (IV-53).

canarone. Hydrogenation of (IV-44) followed by hydrolysis of the ketal and chromatography gave (IV-54) which was pure by VPC on all columns used in this study. Hydrogenation of (IV-28) gave the C-4 methyl epimer (IV-55) which was contaminated with 10 percent of (IV-54) as shown by mixed injection on several VPC columns. Looking over the VPC's of compounds used in making (IV-55), this impurity was detected in all compounds following the second Birch reduction, and it was decided that the methyl epimer is formed in that step.

Side by side comparisons of the IR (Figure 8), NMR (Figure 9), ORD-CD-UV (Figure 10) and mass spectra (Figure 11)of (IV-54) and (IV-55) are shown. The similarities are striking, yet the differences are quite noticeable. Since (IV-54) was pure on five VPC columns, the absorption at 1740 cm^{-1} (see Figure 8a) is due to that compound and cannot readily be explained. It may be due to slowly equilibrating conformers of the molecule. The methyl at C-4 in this molecule is axial, and when the A ring is in a chair configuration a severe one-three interaction exists between it and the methyl at C-10. This might force ring A to exist predominantly in a boat-like or twist-boat-like conformation, which would introduce strain in the ring, causing the carbonyl from this conformer to appear in a region which is usually reserved for somewhat strained carbonyls.[105] The methyl epimer (IV-55) shows only an unstrained carbonyl absorption (1710 cm^{-1}). As is clearly evident, differences do occur in the region from 1500 cm^{-1} to 1200 cm^{-1} for the two

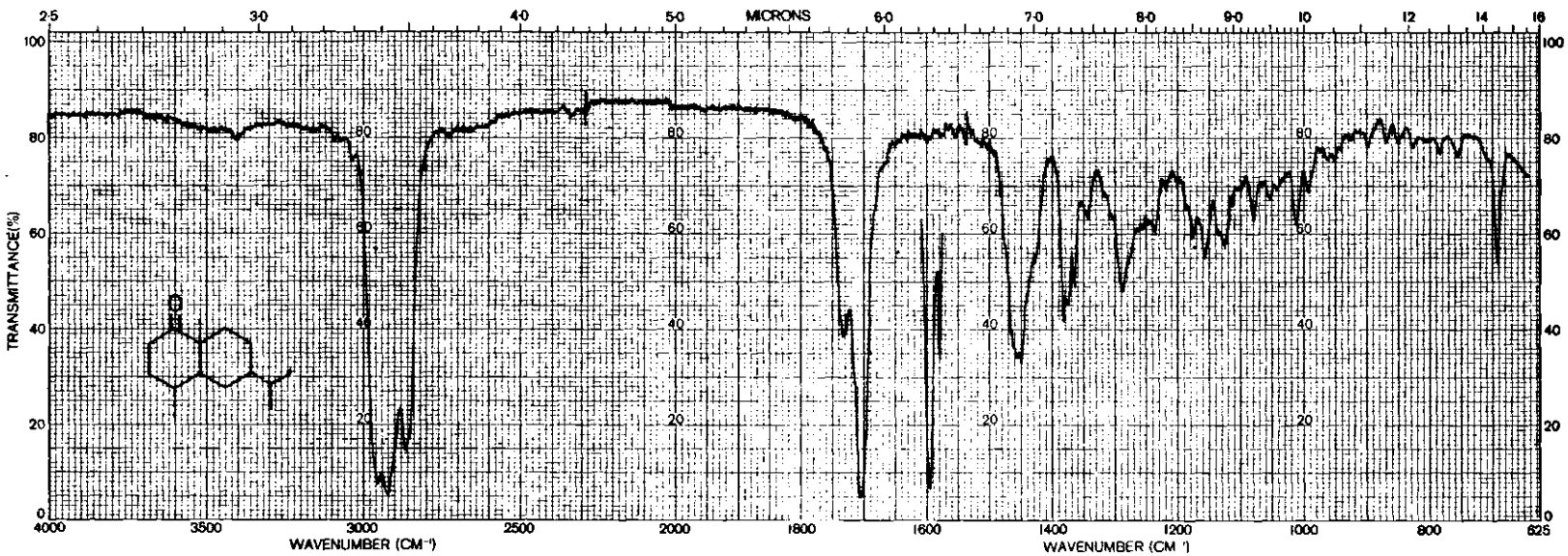


Figure 8a. IR of (IV-54).

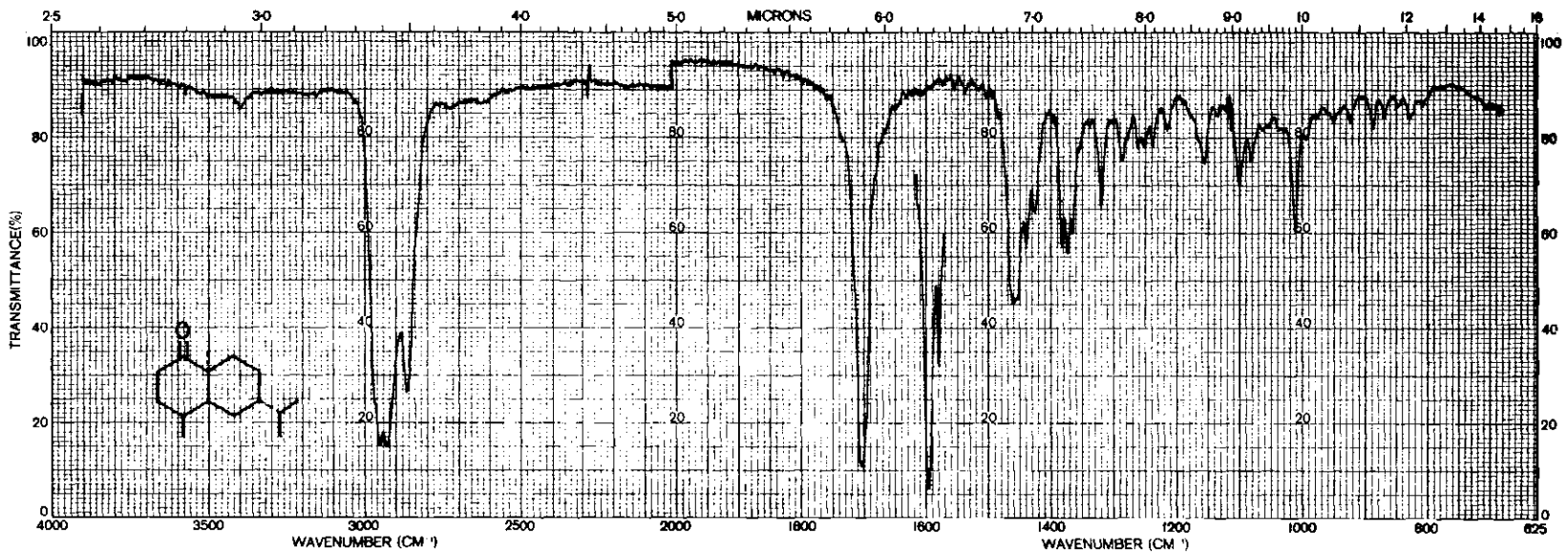


Figure 8b. IR of (IV-55).

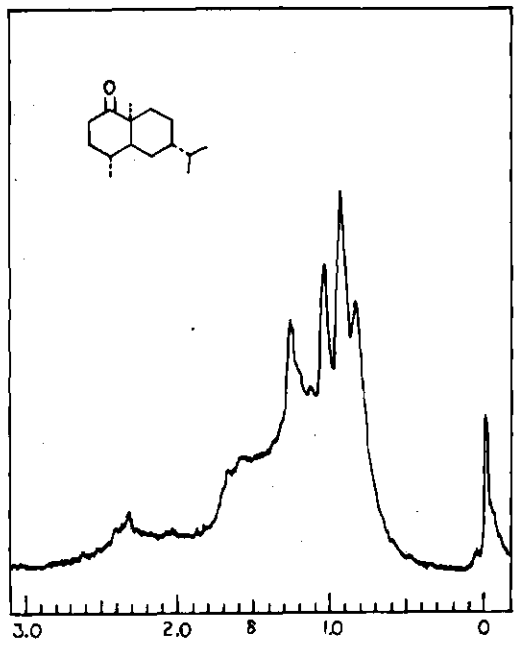


Figure 9a. NMR of (IV-54).

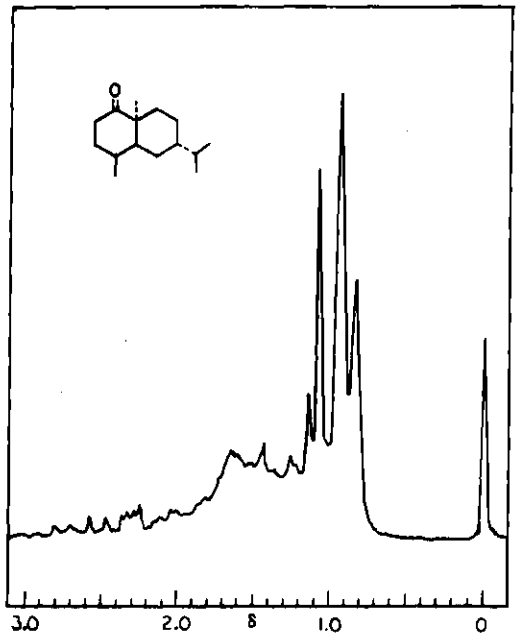


Figure 9b. NMR of (IV-55).

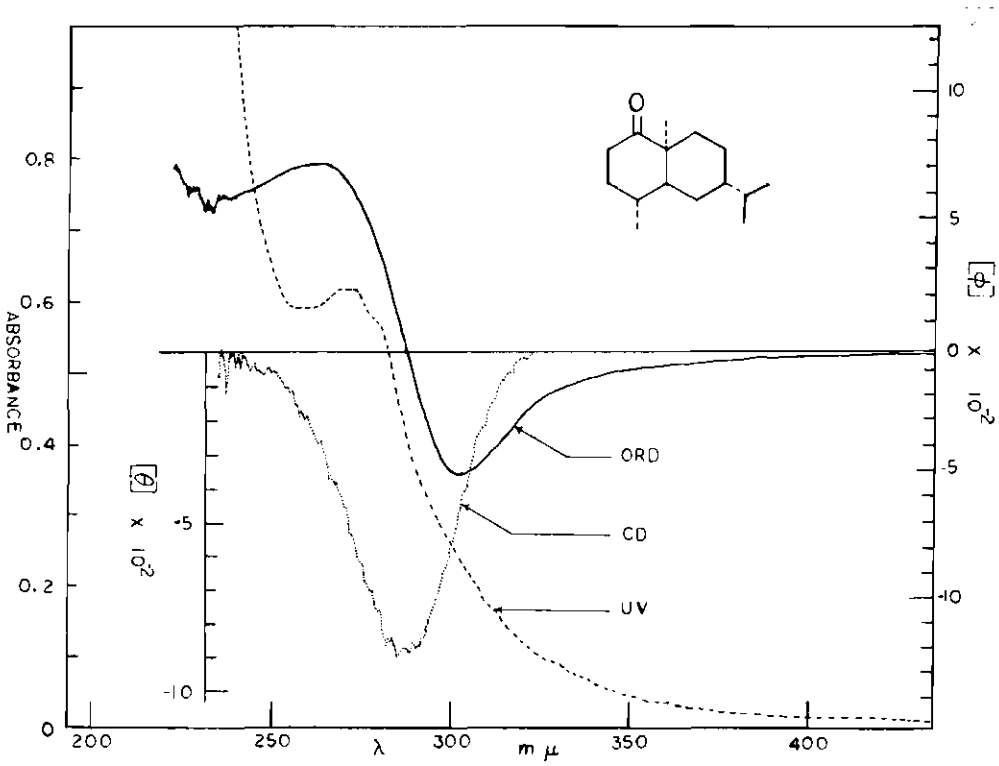


Figure 10a. ORD-CD-UV of (IV-54).

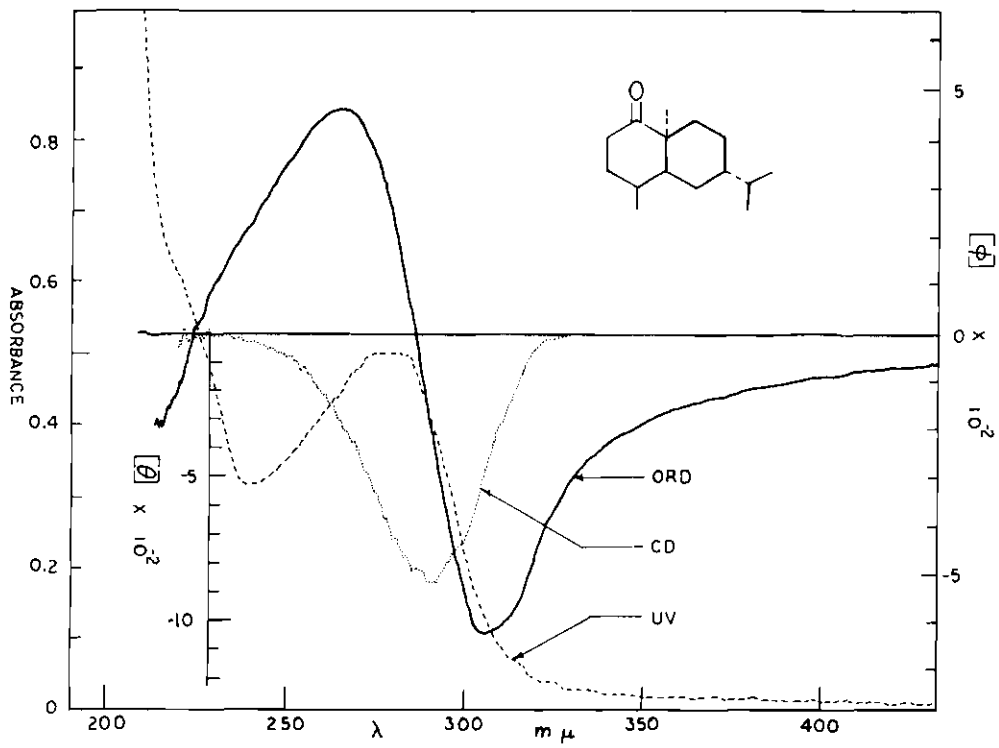


Figure 10b. ORD-CD-UV of (IV-55).

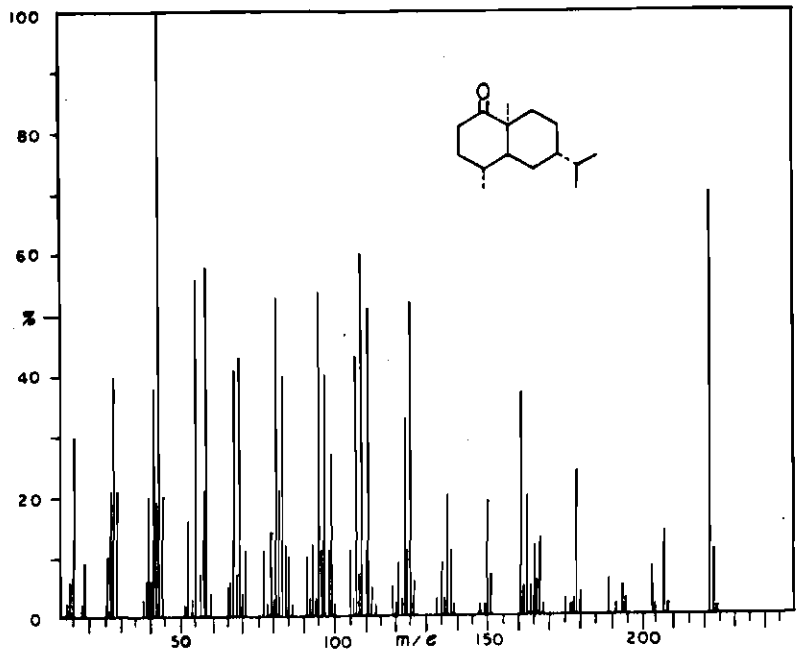


Figure 11a. Mass Spectrum of (IV-54).

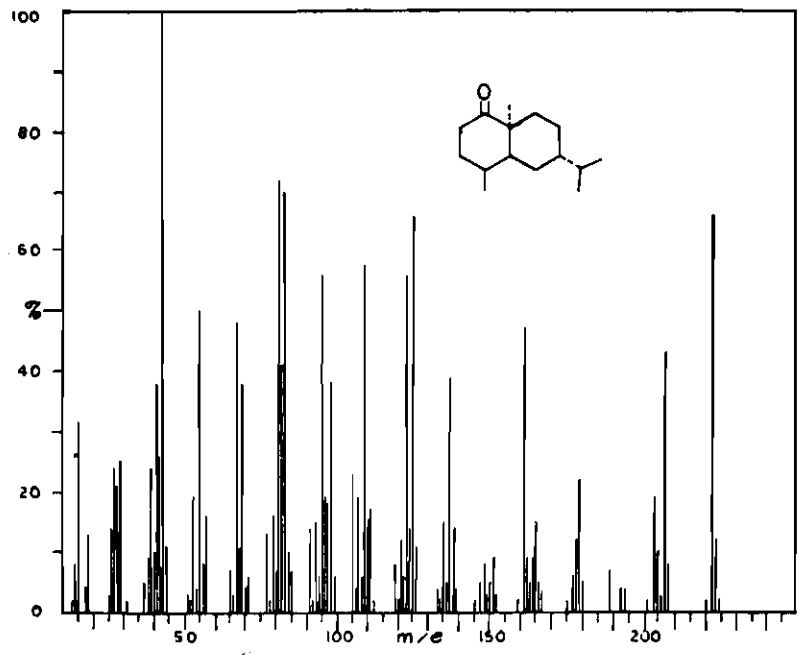


Figure 11b. Mass Spectrum of (IV-54).

epimers. In the NMR of (IV-54) (Figure 9a) the angular methyl absorption and the isopropyl methyl absorptions are separated, and the C-4 methyl absorption appears as a poorly resolved doublet at δ1.23. For (IV-55) (Figure 9b), all the methyl absorptions appear at higher field.

Stereochemical differences are best noted in the ORD-CD curves which are reproduced in Figure 10. Since (IV-54) is a strained system, it might be expected to show a higher amplitude in the ORD curve. It does, but only slightly (a = -12 versus a = -11 for (IV-55)). However, the change in substituent is at C-4, a position which offers no contribution to the Cotton effect.[118] Both curves show the expected negative Cotton effect curve for the trans fused ring system of the indicated absolute configuration.[118] If (IV-54) existed with the A ring predominantly in a boat configuration, the sign of the Cotton effect would still be negative. However, there is a positional shift of some of the substituents in the positive octants which might change the amplitude and shape slightly when going from (IV-55) to (IV-54). Hence, it is felt that differences between the two ORD curves are indicative of the boat configuration of the A ring of (IV-54). There is also a shift to lower wavelength in the $n \rightarrow \pi^*$ transition when going from (IV-54) to (IV-55) (267 mμ to 277 mμ), and the ease of transition (the extinction coefficient) is higher in (IV-54) than (IV-55) (206 versus 183).

An investigation of the mass spectra (Figure 11) is interesting in that both compounds give the same fragmentation pattern; however, very significant differences in relative abundances are observed. This

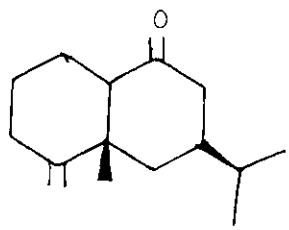
is quite reasonable since the molecules are basically identical, the only difference being at C-4. This should not be enough to inhibit a fragmentation pattern completely, but should be enough to change the probability of it occurring.

Let us now speculate on the structure of canarone as related to this study. Zalkow and Lacoume have shown that (IV-2) is not the correct structure for canarone.[95] Our first indication that (IV-1) is not the correct structure for canarone can be found in comparing our IR spectrum of (IV-54) with that reported for dihydrocanarone. Bhattacharyya reports a carbonyl absorption at 1704 cm^{-1} for dihydrocanarone. For (IV-54) we have, as discussed previously, two bands in the carbonyl region at 1730 cm^{-1} and 1700 cm^{-1}, clearly different from that reported for dihydrocanarone.

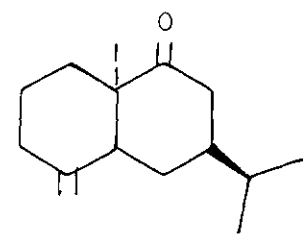
Now let us compare the spectra of (IV-53) and the spectra reported for canarone. Turning first to the NMR spectra (see Figure 5) we see that Bhattacharyya shows a methyl absorption for the angular methyl at δ1.27. On the NMR spectrum of (IV-53) a peak appears in this region, but the angular methyl appears as a shoulder on one of the absorptions of the isopropyl doublet. The big difference in the NMR is the methylene region. For canarone, almost all the methylene protons appear in the region between δ1.7 and δ3.0. For (IV-53), the methyl region is divided into two parts, δ1.0 to δ1.6 (mostly B ring protons) and δ2.0 to δ3.0 (mostly A ring protons). This is clearly different from natural canarone. Differences in the IR also appear. Peak by peak comparison is difficult since

canarone's IR was recorded on a Perkin-Elmer 137B (linear in microns) and (IV-53) on a Perkin-Elmer 237B (linear in cm^{-1}). However, several differences appear. Canarone has a carbonyl absorption at 1700 cm^{-1} and (IV-53) at 1720 cm^{-1}. The double bond absorption is at 1640 cm^{-1} for canarone versus 1660 cm^{-1} for (IV-53). The isopropyl doublet appears at 1375 and 1360 cm^{-1} for canarone and at 1380 and 1375 cm^{-1} for (IV-53). Finally there are differences in the shapes of the ORD curves of canarone and (IV-53) (see Figure 7), again emphasizing the differences between (IV-53) and canarone.

Since (IV-1) and (IV-2) are clearly not correct structures for canarone, let us speculate on some likely possibilities for its structure. Since dehydration over selenium yielded naphthalenic products, a decalone system should be the basic unit of the structure. The NMR of canarone has only low field methylene absorptions, while (IV-53) has both high field and low field methylene absorptions. Probably the carbonyl group and the exocyclic methylene group of canarone are on different rings. Since the two likely eudesmane possibilities suggested by Bhattacharyya are not correct, a likely possibility is an eremophilone structure such as (IV-56). Another possibility is an intermedeol-like structure such as (IV-57). Both



IV-56



IV-57

of these structures would fit the published data and are biogenetically likely.

Indications toward the structure of canarone can be found by taking its mass spectrum. In Figure 12 are shown the mass spectra for (IV-53) and (IV-2) as synthesized by Zalkow and Lacoume.[95] If (IV-57) is the correct structure for canarone, the mass spectral fragmentation pattern should resemble the one shown in Figure 12a. Should the carbonyl group be in the A ring, the fragmentation pattern should resemble that shown in Figure 12b. Unfortunately, the lack of fragmentation directing groups prevents simple analysis of the fragmentation pattern.[127] However, the rough comparison suggested should solve some ambiguity in canarone's structure.

In summary, canarone has been reported to have structure (IV-1) and (IV-2). Synthesis of (IV-2), as its enantiomer (IV-53), has now been accomplished and it is not the correct structure for canarone. Two biogenetically likely structures for canarone have been proposed ((IV-56 and (IV-57)). Hopefully, future work will solve the structure of canarone.

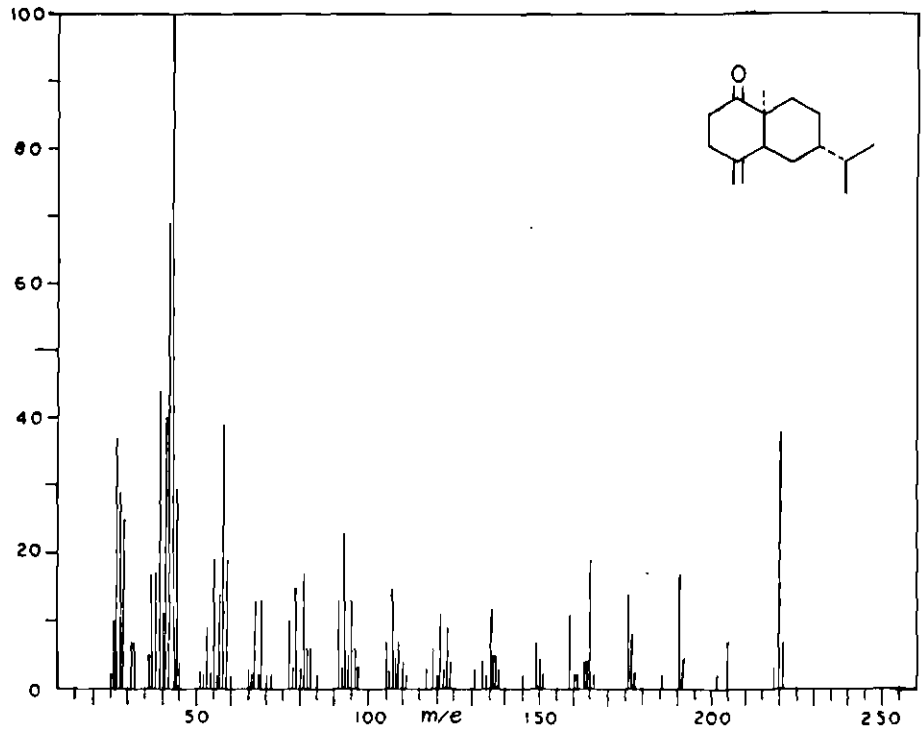


Figure 12a. Mass Spectrum of (IV-53).

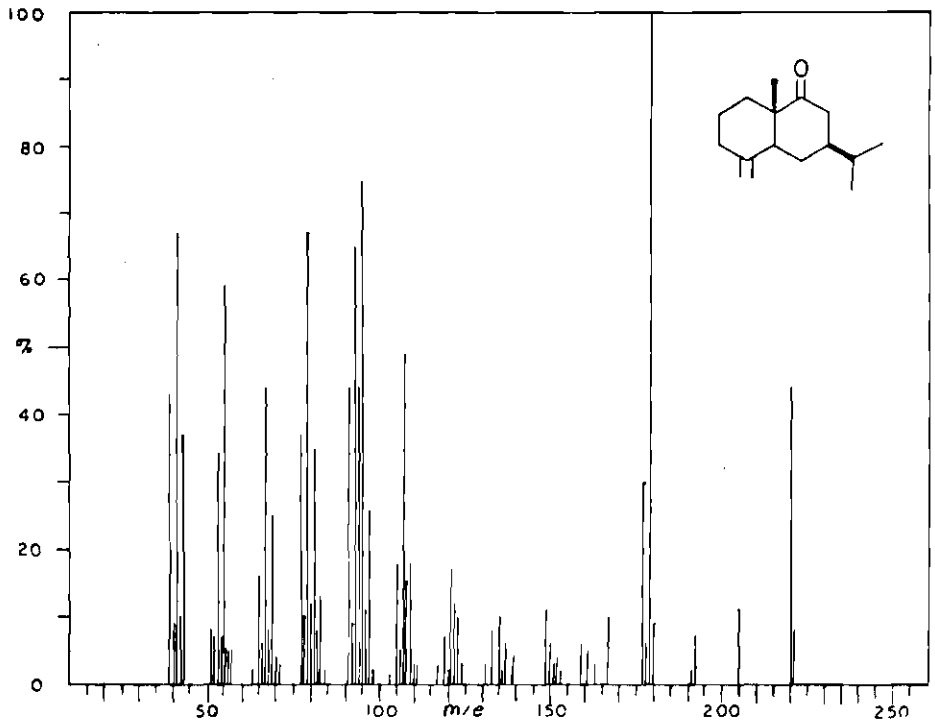


Figure 12b. Mass Spectrum of (IV-2) as Prepared by Zalkow and Lacoume[95].

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The structures of three natural products have been investigated. We have found the structure of one isolated from a plant, revised the structure of another, and have hopefully reopened investigation into the structure of canarone.

We have found that the major constituent of the KB active fraction of Sarracenia flava is a well known triterpene acid, betulinic acid.[47] Hopefully, further work will be done in order to isolate the active compound or compounds that are in this plant. A complete chemical study of the composition of this plant will be very informative since it is a carnivorous plant. It is hence dependent on insects for many of its necessary nutrients, especially nitrogen. Because of this, the metabolic pathways of the plant may be quite different from those in previously studied plants, especially those involved in the biosynthesis of the nitrogen-containing alkaloids. Since an isoprenoid compound has been isolated, it is now known that this metabolic pathway, which was discussed in the first chapter, exists in this plant. Which other interesting compounds will be isolated remains to be seen.

Toxol, a compound isolated by Zalkow and co-workers,[36] has now had its structure revised by the work of E. Keinan[89] and of this thesis. While the revision solves an ambiguity in the use

of Karplus's equation[71] as applied to benzofurans, it does, however, bring up a question as to the usefulness of ozonolysis as a structural determination tool for benzofurans. Keinan has studied the ozonolysis of several benzofurans and has reported that he obtained the expected products.[89] His work makes a repeat of the ozonolysis experiment on toxol even more important.

The structure first proposed by Bhattacharyya for canarone[24] has been found to be incorrect. While this structure is biogenetically quite probable, it is not canarone. Studies into the structure of canarone should be reopened as it is possible that it may have an interesting eremophilone or intermedeol structure. If the isolation of canarone is not too tedious a process, it should be repeated. The mass spectrum should be taken in order to give some idea as to whether further synthetic studies should be directed toward the eremophilone or the intermedeol structure.

Finally, several interesting outgrowths of this thesis work should be pursued. First, one should find out if the biosynthesis of the decalane sesquiterpenes (eudesmanes, eremophilanes, etc.) proceeds through an epoxide mechanism similar to the steroids and triterpenes. If it does, which epoxide is needed for synthesis? Secondly, biogenetic studies into the isoprenoid benzofurans should be undertaken as this is a hazy area of chemical knowledge. Thirdly, a more systematic study of the use of N-bromosuccinimide as a bromination reagent should be undertaken in order to outline its limitations. Fourthly, an extensive mass spectral study of the fragmentation mechanism of sesquiterpenes should be undertaken in

order to extend the usefulness of this tool into the area of sesquiterpene structure. Hopefully many future chemical studies will be directed into these areas.

BIBLIOGRAPHY

1. F. Wöhler, Poggend. Annal., 13, 488 (1828).

2. R. B. Woodward, "Synthesis" in Perspectives in Organic Chemistry, A. R. Todd, ed., Interscience, New York (1956).

3. A. Eschenmosher, "Studies on Organic Synthesis" in XXIIIrd International Congress of Pure and Applied Chemistry, Vol. 2, Butterworth's, London, Eng. (1971), p. 69.

4. L. Ruzicka, Experientia, 9, 357 (1953).

5. Verley, Bull. Soc. Chim., France, 25, 68 (1919).

6. J. S. Simonsen and L. N. Owen, The Terpenes, Vol. I, 2nd ed., Cambridge University Press, Cambridge, Eng. (1947), p. 370.

7. J. S. Simonsen and L. N. Owen, The Terpenes, Vol. II, 2nd ed., Cambridge University Press, Cambridge, Eng. (1949), p. 105.

8. E. J. Corey, N. Ohno, P. A. Vatakenchevy and R. B. Mitra, J. Amer. Chem. Soc., 86, 1478 (1964).

9. R. B. Bates, D. M. Gale and B. J. Gruner, J. Org. Chem., 28, 1086 (1963) and references therein.

10. E. J. Corey and E. Hamanka, J. Amer. Chem. Soc., 89, 2758 (1967) and references therein.

11. a) J. S. Simonsen and D. H. R. Barton, The Terpenes, Vol. III, Cambridge University Press, Cambridge, Eng. (1952), p. 92.
    b) R. H. Moffett and D. Rogers, Chem. and Ind., 916 (1953).

12. R. M. Coates and R. M. Freidinger, Chem. Commun.,871 (1969).

13. J. A. Marshall and M. T. Pike, Tetrahedron Lett., 3107 (1965) and references therein.

14. L. H. Zalkow, V. B. Zalkow and D. R. Brannon, Chem. and Ind., 38 (1963).

15. E. J. Eisenbraun, T. George, B. Riniker and C. Djerassi, J. Amer. Chem. Soc., 82, 3648 (1960).

16. E. J. Corey and E. A. Broger, Tetrahedron Lett., 1779 (1969) and references therein.

17. L. H. Zalkow, F. X. Markely and C. Djerassi, J. Amer. Chem. Soc., 82, 6354 (1960) and references therein.

18. R. J. McClure, K. S. Schorno, J. A. Bertrand and L. H. Zalkow, Chem. Commun., 135 (1968).

19. a) E. J. Corey, W. E. Russey and P. R. Ortiz deMontellano, J. Amer. Chem. Soc., 88, 4750 (1966).
    b) E. J. Corey and W. E. Russey, J. Amer. Chem. Soc., 88, 4751 (1966).
    c) E. E. vanTamelen, J. D. Willet, R. B. Clayton and K. E. Lord, J. Amer. Chem. Soc.,88, 4752 (1966).
    d) J. D. Willet, K. B. Sharpless, K. E. Lord, E. E. vanTamelen and R. B. Clayton, J. Biol. Chem., 242, 2182 (1967).
    e) L. J. Mulherin and P. J. Ramm, Chem. Soc. Rev., 1, 259 (1972).

20. a) R. B. Clayton, Quart. Rev., 19, 168, 201 (1965).
    b) E. E. vanTamelen, R. P. Hanylick, R. B. Clayton and A. L. Burlingame, J. Amer. Chem. Soc., 92, 2137 (1970).
    c) M. Jayme, P. C. Schafer and J. H. Richards, J. Amer. Chem. Soc., 92, 2059 (1970).
    d) E. E. vanTamelen and J. H. Freed, J. Amer. Chem. Soc., 92, 7206 (1970).

21. A. L. Lehninger, Biochemistry, Worth Pub., Inc., New York (1970), p. 204.

22. W. Parker, J. S. Roberts and R. Ramage, Quart. Rev., 21, 331 (1967).

23. D. F. MacSweeney, R. Ramage and A. Satter, Tetrahedron Lett., 557 (1970).

24. A. D. Wagh, S. K. Paknikar and S. C. Bhattacharyya, J. Org. Chem., 29, 2479 (1964).

25. R. Robinson, cited in A. R. Penfold and J. S. Simonsen, J. Chem. Soc., 87 (1939).

26. W. D. MacLeod, Tetrahedron Lett., 4779 (1965).

27. a) C. H. Heathcock and T. R. Kelly, Tetrahedron, 24, 3753 (1968).
    b) G. Mehta, G. L. Chetty, U. R. Nayak and S. Dev, Tetrahedron, 24, 3775 (1968).
    c) H. Hikino, T. Kohama and T. Takemoto, Tetrahedron, 25, 1037 (1969).
    d) J. W. Huffmann, J. Org. Chem., 37, 2736 (1972).

28. P. Karlson, Introduction to Modern Biochemistry (translated by C. H. Doering), 3rd ed., Academic Press, New York (1968), p. 262.

29. a) H. C. Rolling, J. Mol. Biol., 241, 3233 (1966).
b) E. E. vanTamelen and M. A. Schwartz, J. Amer. Chem. Soc., 93, 1780 (1971).
c) L. J. Altman, R. C. Kowerski and H. C. Rolling, J. Amer. Chem. Soc., 93, 1782 (1971).
d) H. C. Rolling, C. D. Poulter, W. W. Epstein and B. Larsen, J. Amer. Chem. Soc., 93, 1783 (1971).
e) R. M. Coates and W. H. Robinson, J. Amer. Chem. Soc., 93, 1785 (1971).

30. a) W. S. Johnson, Acc. Chem. Res., 1, 1 (1968) and references therein.
b) W. S. Johnson, M. B. Gravestock, R. J. Parry, R. F. Meyers, T. A. Bryson and D. H. Miles, J. Amer. Chem. Soc., 93, 4330 (1970).
c) W. S. Johnson, "Recent Developments in Non-enzymatic Biogenetic-like Olefinic Cyclizations," International Symposium on Organic Synthesis, Vancouver, B. C. (1972).
d) E. E. vanTamelen, Acc. Chem. Res., 1, 111 (1968) and references therein.
e) K. B. Sharpless and E. E. vanTamelen, J. Amer. Chem. Soc., 91, 1848 (1969).
f) E. E. vanTamelen, G. M. Milne, M. I. Suffness, M. C. Rudler, R. J. Anderson and R. S. Achini, J. Amer. Chem. Soc., 92, 7202 (1970).
g) E. E. vanTamelen and J. W. Murphy, J. Amer. Chem. Soc., 92, 7204 (1970).
h) E. E. vanTamelen and R. J. Anderson, J. Amer. Chem. Soc., 94, 8225 (1972).
i) E. E. vanTamelen, R. A. Holton, R. E. Hopla and W. E. Kong, J. Amer. Chem. Soc., 94, 8228 (1972).
j) E. E. vanTamelen, M. P. Sieler and W. Wierenga, J. Amer. Chem. Soc., 94, 8229 (1972).

31. J. S. Simonsen and W. C. Ross, The Terpenes, Vol. V, Cambridge University Press, Cambridge, Eng. (1957), p. 318.

32. J. H. Birkinshaw, P. Chaplen and W. P. K. Findley, Biochem. J., 66, 188 (1957).

33. G. A. Shaw, An Introduction to the Alkaloids, John Wiley and Sons, Inc., New York (1967), p. 127.

34. T. Asao, G. Büchi, M. M. Abdel-Kasler, S. B. Chang, E. L. Wick and G. N. Wogen, J. Amer. Chem. Soc., 87, 882 (1965).

35. F. M. Dean, Naturally Occurring Oxygen Ring Compounds, Butterworth's, London (1963), p. 135.

36. L. H. Zalkow and N. Burke, Chem. and Ind., 292 (1963).

37. L. H. Zalkow and M. Ghosal, J. Org. Chem., 34, 1646 (1969).

38. M. Biollaz, G. Büchi and G. Miline, J. Amer. Chem. Soc., 92, 1035 (1970).

39. C. T. Ramming, "The Isolation and Synthesis of 2,5-Diacetylbenzofuran," M. S. Thesis, Oklahoma State University (1963).

40. a) L. H. Zalkow, N. I. Burke and G. Keen, Tetrahedron Lett., 217 (1964).
b) L. H. Zalkow, G. A. Cabot, G. L. Chetty, M. Ghosal and G. Keen, Tetrahedron Lett., 5727 (1968).

41. Private communication to Dr. L. H. Zalkow from local residents who wish to remain anonymous.

42. Dr. S. K. Gabriel, private communication.

43. M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, J. Amer. Chem. Soc., 88,3888 (1966).

44. D. H. Miles, "The Synthesis of Tetracyclic Diterpenoid Alkaloids and Related Diterpenoids," Ph.D. Thesis, Georgia Institute of Technology (1969).

45. International Tables for X-ray Crystallography, Vol. I, H. F. M. Henry and K. Lonsdale, eds., Kynoch Press, Birmingham, Eng. (1952).

46. L. F. Fieser and M. Fieser, Reagents for Organic Synthesis, Vol. I, John Wiley and Sons, Inc., New York (1968), p. 142.

47. J. Simonsen and W. C. J. Ross, The Terpenes, Vol. VI, Cambridge University Press (1957).

48. Dr. R. L. Hale, private communication.

49. Dr. J. B. Nabors, private communication.

50. Dr. D. H. Miles, private communication.

51. J. F. Couch, J. Agr. Res., 35, 547 (1927).

52. J. F. Couch, J. Amer. Med. Assoc., 91, 234 (1928).

53. J. F. Couch, J. Agr. Res., 40, 649 (1930).

54. J. F. Couch, J. Amer. Chem. Soc., 51, 3617 (1929).

55. a) O. C. Dermer and R. Cleverdon, Proc. Okla. Acad. Sci., 23, 63 (1943).
b) S. O. Butler, "Fractions of Tremetol and Their Toxicities," M. S. Thesis, Oklahoma State University (1945).

56. W. A. Bonner, J. I. DeGraw, D. M. Bowen and V. R. Shah, Tetrahedron Lett., 417 (1961).

57. W. A. Bonner and J. I. DeGraw, Tetrahedron, 18, 1295 (1962).

58. L. H. Zalkow, N. Burke, G. Cabot and E. A. Grula, J. Med. Pharm. Chem., 5, 1342 (1962).

59. J. I. DeGraw and W. A. Bonner, Tetrahedron, 18, 1311 (1962).

60. J. I. DeGraw and W. A. Bonner, J. Org. Chem., 27, 3917 (1962).

61. J. I. DeGraw, D. M. Bowen and W. A. Bonner, Tetrahedron, 19, 19 (1963).

62. D. M. Bowen, J. I. DeGraw, V. R. Shah and W. A. Bonner, J. Med. Chem., 6, 315 (1963).

63. B. Kamthong and A. Robertson, J. Chem. Soc., 925 (1939).

64. N. Burke, "An Investigation of the Toxic Plant - Rayless Goldenrod," Ph.D. Thesis, Oklahoma State University (1965).

65. W. A. Bonner, N. I. Burke, W. E. Fleck, R. K. Hill, J. A. Joule, B. Sjöberg and L. H. Zalkow, Tetrahedron, 20, 1419 (1964).

66. C. Djerassi, Optical Rotatory Dispersion, McGraw-Hill, New York (1960).

67. G. Büchi, L. Crombie, P. J. Godin, J. S. Kaltenbronn, K. S. Siddalingaiah and D. A. Whiting, J. Chem. Soc., 2843 (1961).

68. M. Nakayaki and H. Arakawa, Bull. Chem. Soc., Japan, 34, 1246 (1961).

69. P. K. Ramachandran, T. Cheng and W. J. Horton, J. Org. Chem., 28, 2744 (1963).

70. L. H. Zalkow and M. Ghosal, Chem. Commun., 922 (1967).

71. M. Karplus, J. Chem. Phys., 30, 11 (1959).

72. M. Karplus, J. Amer. Chem. Soc., 85, 2870 (1963).

73. H. Booth, Tetrahedron Lett., 411 (1965).

74. D. P. Brust, D. S. Tarbell, S. M. Hecht, E. C. Hayward and L. D. Colebrook, J. Org. Chem., 31, 2192 (1966).

75. E. C. Hayward, D. S. Tarbell and L. D. Colebrook, J. Org. Chem., 33, 399 (1968).

76. S. P. Pappas, R. D. Zehr and J. E. Alexander, J. Hetero. Chem., 7, 1215 (1970).

77. M. P. Mertes, L. J. Powers and E. Shefter, Chem. Commun., 620 (1970).

78. M. J. Buerger, The Precession Method, John Wiley and Sons, Inc., New York (1964).

79. D. W. Beard, "Goniostat Alignment Instructions," Picker Instruments, Cleveland, Ohio (1967).

80. F. Carter, "Master Card Program for the Picker Four-Angle Programmer," Picker Instruments, Cleveland, Ohio (1967).

81. Programs used for calculations were A. Zalkin, "Fortran Fourier Analysis Program, FORDAP;" W. R. Busing, K. O. Martin and H. A. Levy, "Fortran Crystallographic Least Squares Program (XFLS), Modified Version of ORFLS;" and various locally written programs. All calculations were performed on a UNIVAC 1108 computer.

82. A. L. Patterson, Z. Krist., A90, 517 (1935), also see M. J. Buerger, Vector Space, John Wiley and Sons, Inc., New York (1959) for a more recent discussion.

83. D. Harker, J. Chem. Phys., 4, 381 (1936).

84. International Tables for X-ray Crystallography, Vol. III, C. H. MacGillavary, G. D. Rieck and K. Lonsdale, eds., Kynoch Press, Birmingham, Eng. (1962).

85. G. H. Stout and L. H. Jenson, X-ray Structure Determination, MacMillan Co., New York (1968), p. 245-246. $R = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$.

86. ibid., p. 385-397.

87. C. K. Johnson, "Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, ORTEP," Oak Ridge National Laboratories.

88. W. C. Hamilton, Science, 169, 133 (1970).

89. E. Keinan, "The Absolute Configuration of Toxol," M. S. Thesis, Weizmann Institute, Rehovoth, Israel (1972).

90. L. H. Zalkow, E. Keinan, S. Steindel, A. R. Kalyanaraman and J. A. Bertrand, Tetrahedron Lett., 2873 (1972).

91. H. O. House, Modern Synthetic Reactions, 2nd ed., W. A. Benjamin, Inc., Menlo Park, Calif. (1972), p. 54-70.

92. G. Büchi, M. S. Wittenau and D. M. White, J. Amer. Chem. Soc., 81, 1968 (1959).

93. G. S. K. Rao and S. Dev, J. Indian Chem. Soc., 33, 561 (1956).

94. V. K. Hinge, A. D. Wagh, S. K. Paknikar and S. C. Bhattacharyya, Tetrahedron, 21, 3197 (1965).

95. B. Lacoume and L. H. Zalkow, Tetrahedron Lett., 5881 (1966).

96. E. E. Blaise and M. Maire, Bull. Chim. Soc., 3, 543 (1908).

97. F. J. McQuillin, J. Chem. Soc., 528 (1955).

98. D. W. Theobald, Tetrahedron, 21, 791 (1965).

99. G. Mehta, G. L. Chetty, U. R. Nayak and S. Dev, Tetrahedron, 24, 3775 (1968).

100. R. Howe and F. J. McQuillin, J. Chem. Soc., 2670 (1956).

101. J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, J. Chem. Soc., 1094 (1952).

102. P. J. Kropp and H. J. Krauss, J. Amer. Chem. Soc., 89, 5199 (1967).

103. P. E. Eaton and T. W. Cole, J. Amer. Chem. Soc., 86, 962 (1964).

104. H. J. Dauben and L. L. McCoy, J. Org. Chem., 24, 1577 (1959).

105. J. R. Dyer, Applications of Absorption Spectroscopy of Organic Compounds, Prentice-Hall, Inc., Englewood Cliffs, N. J., (1965).

106. F. W. McLafferty, Interpretation of Mass Spectra, W. A. Benjamin, Inc., New York (1966).

107. J. Ringold and S. K. Malhotra, Tetrahedron Lett., 669 (1962).

108. H. O. House, M. Gall and H. D. Olmstead, J. Org. Chem., 36, 2361 (1971).

109. T. G. Halsall, D. W. Theobald and K. B. Walshaw, J. Chem. Soc., 1029 (1964).

110. J. A. Marshall and W. I. Fanta, J. Org. Chem., 29, 2501 (1964).

111. A. G. Hortmann, J. E. Martinelli and Y. Wang, J. Org. Chem., 34, 732 (1969).

112. E. L. Eliel, Stereochemistry of Carbon Compounds, McGraw-Hill Book Co., Inc., New York (1962).

113. H. O. House, Modern Synthetic Reactions, Chapter 3.

114. a) P. S. Wharton and D. H. Bohlen, J. Org. Chem., 26, 315 (1961).
b) P. S. Wharton, J. Org. Chem., 26, 4781 (1961).

115. Dr. N. G. Schnautz, private communication.

116. H. O. House and B. M. Trost, J. Org. Chem., 30, 2502 (1965).

117. M. P. Hartshorn and E. R. H. Jones, J. Chem. Soc., 1312 (1962).

118. P. Crabbé, Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, Holden-Day, Inc., San Francisco (1965).

119. a) C. A. Bunton and G. J. Minkoff, J. Chem. Soc., 665 (1949).
b) H. B. Henbest, W. R. Jackson and I. Malunowicz, J. Chem. Soc., C, 2469 (1967).

120. D. G. Lee, "Oxidation of Oxygen - and Nitrogen - Containing Functional Groups with Transition Metal Compounds," in Oxidation, Vol. 1, R. L. Augustine, ed., Marcel Dekker, Inc., New York (1969).

121. H. O. House, Modern Synthetic Reactions, Chapter 9.

122. L. H. Zalkow and G. L. Chetty, J. Org. Chem., submitted.

123. J. A. Marshall, Acc. Chem. Res., 2, 33 (1969).

124. S. N. Lewis, "Peracid and Peroxide Oxidations," in Oxidation, Vol. 1, R. L. Augustine, ed., Marcel Dekker, Inc., New York (1969).

125. J. March, Advanced Organic Chemistry, McGraw-Hill Book Co., New York (1968).

126. R. L. Augustine, Catalytic Hydrogenation, Marcel Dekker, Inc., New York (1965).

127. H. Budzikiewicz, C. Djerassi and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Vol. II, Holden-Day, Inc., San Francisco (1964).

VITA

Steven Jay Steindel was born on September 20, 1948 to Marvin and Ruth Steindel in Brooklyn, New York. He has one brother, Charles, three years younger than he.

Steven attended elementary and junior high school in the public schools of New York City. He attended Brooklyn Technical High School for two years, specializing in chemistry.

In 1965, Steven's family moved to Atlanta, Georgia where they now reside. Steven graduated from Briarcliff High School in Atlanta, Georgia, with honors, in June, 1966.

Steven then attended the Georgia Institute of Technology where he received his Bachelor of Science in Chemistry degree in June, 1970. He remained there in order to complete the requirements for a Ph.D. in chemistry.

After completion of his degree requirements, Steven will join Dr. M. G. Rossmann at Purdue University to study the structure and function of dehydrogenases. He has been awarded National Institute of Health and American Cancer Society Postdoctoral Fellowships for this work.

Steven is currently engaged to Miss Stephanie Tsivoglou of Atlanta, Georgia. They are to be married on March 24, 1973.